Low-Molecular-Weight Heparins and Heparinoids and their Use in Acute or Progressing Ischemic Stroke

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Summary: Thrombotic or thromboembolic occlusion of a cerebral artery is the most common pathophysiologic mechanism of acute ischemic stroke. An antithrombotic agent would therefore appear to be an ideal medication for treatment of this condition. Heparin is an effective anticoagulant, but it has poor bioavailability and effects on thrombin and platelets that predispose it to life-threatening complications such as hemorrhage and thrombocytopenia. Low-molecular-weight (LMW) heparins have better bioavailability, a higher anti-Xa:anti-IIa ratio, and less effect on platelets than heparin; yet their heterogeneity has hampered their proper investigation in clinical trials and it has not yet been proven that they exhibit less tendency toward hemorrhage and thrombocytopenia than conventional heparin. The LMW heparin, Org 10172, is superior to standard heparin in terms of its bioavailability, anti-Xa:anti-IIa ratio, and lack of effect on platelets. It is less likely than heparin and many LMW heparins to induce thrombocytopenia. Like the various heparins, Org 10172 exhibits dose-dependent hemorrhagic tendencies, yet preliminary studies have found doses that are safe for use in patients with acute ischemic stroke. These studies also suggest that Org 10172 may improve outcome and lessen mortality in this population. A prospective, randomized, double-blind, controlled trial is needed to establish the potential efficacy of Org 10172 in patients who suffer acute or progressing ischemic stroke. Key Words: Acute ischemic stroke—Heparin—Low-molecular-weight heparins—Heparinoids—Org 10172—Glycosaminoglycans.

Acute ischemic stroke is the leading neurologic reason for hospitalization in the United States. Annually, more than 400,000 Americans have strokes and approximately 150,000 die. Not only is stroke the third most common cause of death in the United States, but it is the leading cause of long-term disability.

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Despite the frequent occurrence of ischemic stroke, no therapy has been established as effective in acute management. Treatment is presently restricted to rehabilitation and prevention of medical complications. Although some experiments suggest that early intervention, i.e., within the first few hours after onset, may ameliorate or reverse the process of acute brain ischemia, no such treatment has been shown to be efficacious in humans.

THE PROBLEM: ISCHEMIC STROKE AND HEPARIN THERAPY

An early and effective treatment of acute ischemic stroke is needed. One of the most frustrating aspects of the management of an acute ischemic stroke is that approximately 25–40% of patients will deteriorate after admission to the hospital (1–3). While neurologic worsening can occur up to several days following stroke, most instances of stroke progression occur within the first 24 h. Duke et al. (3) reported progression during the first 24 h of stroke in 28.2% of patients. The outcomes of patients who deteriorate after admission are poorer than those who do not worsen and, unfortunately, one cannot predict which patients will worsen. Progression can develop with any subtype of ischemic stroke; therefore, all patients with acute ischemic stroke should be considered at risk for worsening.

There are many possible pathophysiologic mechanisms of neurologic worsening, including intracellular or extracellular metabolic changes, development of cerebral edema, recurrent embolism, or extension of an intraluminal thrombus (4). Extension of a thrombus may induce additional occlusion of the artery or failure of collateral channels.

Because acute ischemic stroke is largely due to embolic or thrombotic occlusions of arteries perfusing the brain and because many cases of progressing stroke are secondary to recurrent embolism or extension of thrombi, many physicians believe that the early administration of antithrombotic therapy is an appropriate treatment. Antithrombotic drugs might prevent propagation of a thrombus, forestall distal embolization from a fragmented clot, or halt recurrent embolism from an extracranial source. Antithrombotic drugs might also help maintain collateral circulation to the ischemic area. In addition, antithrombotic drugs could lessen the risk of major complications of stroke such as deep-vein thrombosis and pulmonary embolism.

A drug that has an immediate antithrombotic action would appear to be ideal. For approximately 40 years, heparin has been the most frequently prescribed drug for treatment of patients with acute ischemic stroke and experts in stroke have long recommended its use (5–11). A report from four academic neurologic services with a special interest in stroke confirms the common use of heparin (12). The drug was dispensed during hospitalization to 73% of patients with large-artery atherothrombotic strokes, 54% of patients with cardiogenic strokes, 31% of patients with lacunar strokes, and 38% of patients with infarction of an undetermined etiology.

A 1989 survey of 349 randomly selected neurologists in the United States demonstrated both the widespread use of heparin and the uncertainty regarding its efficacy and safety (13). Approximately 84% of all respondents reported that at

least one of their patients within the previous year had been given heparin for acute ischemic stroke. Overall, they estimated that nearly 22% of their patients with acute stroke received heparin. Despite the widespread use of heparin, only 6.6% of neurologists felt heparin had been shown to be effective in the management of acute ischemic stroke. Conversely, 15.6% believed that heparin had been demonstrated to be ineffective; the remaining neurologists were uncertain. In addition, 42% of neurologists had major concerns about the safety of heparin.

The guidelines for heparin therapy have not been defined and its use in the management of ischemic stroke is very controversial (14–18). Two small randomized trials performed in the 1960s demonstrated a beneficial effect in preventing progression of stroke, but the drug did not reduce mortality (19,20). One small randomized trial found no impact in preventing stroke progression from subcutaneously administered heparin (3). In an uncontrolled study, Haley et al. (21) noted continued progression in 50% of 36 patients with progressing stroke despite “adequate” levels of heparin anticoagulation. Ramirez-Lassepas et al. (22) reported the results of heparin therapy in 150 patients with acute ischemic stroke: 81% had a good recovery, 75% were ambulatory after their stroke, 9% had fluctuations in neurologic deficits, and only 1% worsened.

Furlan et al. (23) reported 54 patients who were treated with heparin for acute cardioembolic stroke: no patients had hemorrhagic transformation of infarctions and 7 patients with inadequate treatment had recurrent embolic events. In a small randomized trial of patients with recent cardioembolic stroke, none of 24 patients given heparin had recurrent embolic events while 2 strokes occurred in 21 patients not given the drug (24). A recent nonrandomized trial among patients with cardioembolic stroke did not demonstrate any differences in the rates of recurrent embolism or hemorrhagic infarction between those who did or did not receive heparin (25).

In a randomized trial, Duke et al. (26) tested the value of continuous intravenous infusions of heparin in preventing progression in 225 patients with acute, stable, partial stroke. Improvement in neurologic condition was noted in 26.6% of patients given heparin and 24.3% of those given placebo. Progression of neurologic deficits was noted in 17% of patients given heparin and in 19.5% of control patients. Unfortunately, this trial had serious limitations. The mean interval between onset of stroke and treatment was 28.4 h among patients given heparin and 25.7 h among those given placebo (27). This delay is important because it means that most patients were treated after the period of greatest risk for continued progression. In addition, patients with cardioembolic or worsening stroke were excluded from the trial. These patients may have been the ones most likely to benefit from heparin treatment. It is hard to agree with the investigators’ conclusion that “early heparin management in patients with acute thrombotic stroke has no benefit on final neurologic deficit” (26) because the design of the trial did not allow for avoidance of a type II statistical error.

Hemorrhage and thrombocytopenia are important complications of heparin and either may result in discontinuation of therapy, actual worsening of neurologic deficit, or even death. Bleeding complications range from mucosal ooze to major visceral or intracranial hemorrhage. In patients of all types treated with
standard-dose heparin, there is a 2–4%/year frequency of major bleeding episodes requiring transfusion (28). In the treatment of deep-vein thrombosis (DVT) and pulmonary embolism, the incidence of major hemorrhages during standard-dose heparin administration is estimated to be approximately 10% (29). Ramirez-Lassepas et al. (30) reported that the risk of intracranial hemorrhage following heparin therapy for acute cerebral ischemia is approximately 0.6% and, for the subset of patients with acute cerebral infarction, the risk is 1.1%. Another study reported hemorrhage into an ischemic area following heparin infusion in 8 of 50 patients with progressing stroke (31). The risk of hemorrhagic transformation of an infarction with the use of heparin is not known because ischemic brain lesions may spontaneously develop hemorrhagic changes (32). The drug’s use, however, may increase the severity of an otherwise asymptomatic transformation.

There is evidence to suggest that heparin’s antithrombotic and hemorrhagic effects can be dissociated and, further, that a major mechanism for the hemorrhagic complications of heparin is its effect on platelet function (29,33,34). This is consistent with the observation that standard high-dose heparin prolongs the bleeding time (29,33). Another mechanism of heparin’s bleeding complications may involve its effects on the coagulation cascade (35,36). Theoretically, the “anticoagulant activity” of heparin (i.e., the in vitro inhibition of coagulation factors as reflected by a prolongation of the activated partial thromboplastin time, or aPTT) is responsible for its bleeding complications and is separate from its “antithrombotic activity” (i.e., the in vivo inhibition of thrombosis).

Another complication associated with heparin treatment is thrombocytopenia (37–43). Combining several prospective studies, it is estimated that 10% (range 0 to 30%) of patients given heparin develop thrombocytopenia (37–39). Among patients with heparin-induced thrombocytopenia, hemorrhage (incidence <10%) is less common than thromboembolism (incidence of 10–20%) (37,38). Two types of heparin-induced thrombocytopenia have been distinguished. In the most common form (type I), the platelet count decreases 1 to 5 days after the onset of heparin therapy, but usually remains greater than 50,000/mm³ and returns to normal despite continued heparin therapy; type I is rarely associated with thromboembolism and is probably caused by a direct heparin-induced platelet aggregation (37). Type II heparin-induced thrombocytopenia is less common but more severe, with a decrease in platelet count to less than 50,000/mm³ (often <10,000/mm³) that generally occurs 6 to 12 days after the onset of heparin therapy, and does not return to normal until several days after the discontinuation of heparin; type II is more often associated with thromboembolism and is probably caused by an IgG and IgM immune-mediated platelet aggregation (37,39–41). The type II reaction may occur earlier in patients previously exposed to heparin; fatal reactions have been reported within 30 min of re-exposure (37). The thrombosis (“white-clot syndrome”) is primarily arterial and often leads to death, myocardial infarction, stroke, or amputation of a limb (37–39,42). In one series, 6 of 18 patients who developed carotid occlusion following carotid endarterectomy had heparin-induced thrombocytopenia (43). In another report, 9 of 14 patients with neurologic deterioration while receiving heparin had declines in platelet counts (40). Thus, some of the actions of heparin may potentiate the progression of the
ischemic stroke process. Part of the failure of heparin in patients with acute ischemic stroke may be related to its effect in promoting rather than inhibiting the thrombotic process.

THE DEVELOPMENT OF LOW-MOLECULAR-WEIGHT HEPARINS AND HEPARINOIDS

Investigators have attempted to discover an agent with the antithrombotic characteristics of heparin but without its hemorrhagic complications. Investigations into heparin's structure and mechanism of action, as well as clinical studies of low-dose vs. full-dose heparin, have led to the development of low-molecular-weight (LMW) heparins and heparinoids.

Heparin is a mixture of acidic, sulfated, linear polysaccharides having average molecular weights of 10,000 to 15,000 Da, with a range of 4,000 to 40,000 Da (44). Although heparin is comprised of a repeating trisulfated disaccharide unit, it exhibits wide structural diversity (Fig. 1). One unique part of its structure is a pentasaccharide sequence containing a 3,6-O-sulfated glucosamine N-sulfate residue; this is the site where heparin binds to the naturally occurring serine-protease inhibitor, antithrombin III (AT III) (45,46). Heparin molecules may be divided into three categories: molecules with low affinity for AT III, high-molecular-weight (>5,000 Da) molecules with high affinity for AT III, and low-molecular-weight (<5,000 Da) molecules with high affinity for AT III (47).

Heparin's antithrombotic activity involves several independent mechanisms of action. Heparin inhibits serine proteases (specifically, coagulation factors IIa, IXa, Xa, XIa, and XIIa) primarily through its binding and activation of AT III (44). The most important antithrombotic effect of heparin–AT III binding is the inhibition of thrombin (factor IIa), both directly and through the inhibition of factor Xa, thus blocking the conversion of fibrinogen to fibrin (47,48). Heparin also acts through heparin cofactor II (HC II), which selectively inactivates thrombin (48). Heparin alters platelet function by inhibition of AT III-independent thrombin-induced platelet aggregation, inhibition of collagen-induced platelet aggregation and collagen–platelet adhesion (possibly through interference with the function of von Willebrand factor), and potentiation of platelet aggregation induced by ADP and other stimuli (with enhanced release of thromboxane A2) (33–35,49,50). Heparin may also have an antithrombotic effect by binding to endothelium (causing release of antithrombotic glycosaminoglycans) and by accelerating fibrinolysis (heparin enhances the activation of plasminogen by tissue plasminogen activator) (29,49–53). Heparin may also have the adverse effect of increasing capillary permeability and microvascular bleeding (54).

Low-dose heparin is effective therapy for thromboembolism prophylaxis with less bleeding complications and less consistent prolongation of the aPTT than full-dose heparin (28,55). This observation, along with the in vitro finding that low doses of heparin inhibit factor Xa and relatively higher doses are required to inhibit thrombin, led to the theory that heparin's hemorrhagic complications are related to antithrombin activity and its antithrombotic properties are related to anti-Xa activity (36,55). The explanation for low-dose heparin's antithrombotic
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FIG. 1. Structure of glycosaminoglycans comprising LMW heparins and heparinoids. The X represents a protein or sulfate group while Y represents an acetate or sulfate group.

Efficacy relates to the amplification mechanism of the coagulation cascade: inhibition of factor Xa results in an exponential increase in the inhibition of factor IIa (35,55). It was subsequently discovered that the high-molecular-weight, high-AT III-affinity molecules of heparin have both anti-IIa and anti-Xa activity while the low-molecular-weight, high-AT III-affinity molecules of heparin have only anti-Xa activity; this led directly to the development of LMW heparins and LMW heparinoids (47,55).

Other studies suggest that a likely explanation for heparin's bleeding complications is its effect on platelet function, particularly the inhibition of platelet
aggregation (29,33–35,50). The effect of heparin on platelets, however, is confined to the molecules of high molecular weight and low affinity for AT III; thus, the LMW heparins and heparinoids (with high affinity for AT III) have less effect on platelets than does heparin, with theoretically less hemorrhagic side effects (33,35).

Heparin is metabolized by the liver and reticuloendothelial system with minimal renal excretion. It does not cross the placenta. Heparin in general exhibits poor bioavailability (56). Intravenously administered heparin acts systemically as an anticoagulant, but, when administered subcutaneously, there is a long delay and only a small percentage of the heparin enters the circulation (56,57). The pharmacodynamics of LMW heparins and heparinoids are different from those of heparin. In addition to displaying enhanced bioavailability when administered subcutaneously, these compounds show a considerably longer in vivo half-life (58,59). Thus, a desire to improve bioavailability and to decrease potentially life-threatening side effects has led to the development of a new generation of antithrombotic agents.

BIOCHEMISTRY, PHARMACOLOGY, AND PREPARATION OF LMW HEPARINS

LMW heparins are modified fractions of heparin, consisting of mixtures of polysaccharides with molecular weights ranging from 2,000 to 8,000 Da (average of 5,000 Da) (34,56,58,60). LMW components comprise less than 10% of commercial heparin.

LMW heparins are generally derived by the controlled chemical or enzymatic depolymerization of heparin (Table 1). Nitrous acid is used to cleave heparin at glucosamine residues containing an N-sulfate group (61). Not all N-sulfated glucosamine residues, however, are equally susceptible to deaminative cleavage. Resistance of 3,6-O-sulfated glucosamine N-sulfate (found in the center of the AT III binding site) leads to the isolation of the AT III-binding oligosaccharides following deaminative cleavage of heparin (62). Thus, partial depolymerization by

<table>
<thead>
<tr>
<th>Agent</th>
<th>Preparation</th>
<th>Avg. MW (Da)</th>
</tr>
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<tbody>
<tr>
<td>CY 216 (Fraxiparin)</td>
<td>EtOH or NA</td>
<td>5,506</td>
</tr>
<tr>
<td>CY 272</td>
<td>Prolonged NA</td>
<td>3,410</td>
</tr>
<tr>
<td>Kabi 2165 (Fragmin)</td>
<td>NA + GPC</td>
<td>6,370</td>
</tr>
<tr>
<td>Sandoz LMWH</td>
<td>AN</td>
<td>6,322</td>
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<tr>
<td>RD heparin</td>
<td>OX</td>
<td>6,221</td>
</tr>
<tr>
<td>OP 2122 (Plasm)</td>
<td>OX</td>
<td>6,311</td>
</tr>
<tr>
<td>PK 10169 (Enoxaparin/Lovenox)</td>
<td>Benzylolation + β-E</td>
<td>3,789</td>
</tr>
<tr>
<td>LHN-1 (Logiparin)</td>
<td>Enzymatic β-E</td>
<td>4,850</td>
</tr>
<tr>
<td>Heparin</td>
<td>PM or BL</td>
<td>14,000</td>
</tr>
<tr>
<td>Org 10172</td>
<td>PM</td>
<td>6,000</td>
</tr>
</tbody>
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* EtOH = ethanol fractionation; NA = nitrous acid depolymerization; GPC = gel-permeation chromatography; AN = isocyanitrile depolymerization; OX = peroxidative depolymerization; β-E = β-elimination; PM = porcine mucosa extraction; BL = bovine lung extraction.

* By GPC (60).
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nitrous acid enriches this site in LMW heparin preparations. Nitrous acid treatment also introduces an artifact into the reducing end of each LMW heparin chain, a ring-contracted anhydromannose residue (60,61). There is no easy way to remove this residue from LMW heparins prepared by nitrous acid cleavage.

Another method of preparing LMW heparins is oxidative cleavage of heparin by Smith degradation. Heparin contains unsulfated glucuronic acid residues that have vicinal diol functionality particularly susceptible to certain oxidants. Cleavage of glucuronic acid results in an acyclic residue that is sensitive to hydrolysis under relatively mild conditions. Because the AT III binding site contains an unsulfated uronic acid, it can be destroyed by this method. Thus, controlled, partial oxidation followed by hydrolysis is required to maintain AT III activity.

Heparin can also be fragmented at uronic acid residues (63). The resulting LMW heparin has an unsaturated iduronic acid residue in the nonreducing end of each chain. This residue may prolong the in vivo half-life of the oligosaccharides by blocking their biotransformation by exoglycuronidases (64). Depolymerization of heparin can also be accomplished enzymatically using heparinase, which breaks heparin's most common linkage, 2,6-disulfated glucosamine-(1→4)-2-sulfated iduronic acid (64). This enzyme is used to prepare LMW heparins with anticoagulant activity (65).

Although each LMW heparin is different and each has artifacts generated in their preparation, they all have structural features in common, as demonstrated by oligosaccharide mapping techniques (60). Additional studies on the structures of these LMW heparin preparations will be required to understand and to rationalize the differences in their biological activity (66).

AT III-mediated actions of LMW heparins have from 5 to 25 times greater anti-Xa activity than antithrombin activity. Stated another way, the anti-Xa:APTT ratio increases with decreasing molecular weight of heparin (36,51). Ex vivo studies of human whole blood have shown that factor Xa inhibition by LMW heparin effectively inhibits thrombin generation, resulting in effective inhibition of fibrin formation; in fact, this indirect inhibition of thrombin by LMW heparin lasted longer than the direct inhibition of thrombin by unfractionated heparin (67). The half-life of LMW heparins is double that of unfractionated heparins (53). In humans, the half-life of LMW heparins is 90 to 150 min after intravenous administration and 200 to 300 min after subcutaneous administration; the corresponding half-lives of standard heparin are 50–60 and 100–120 min (36). The bioavailability of LMW heparin is greater than that of standard heparin—approximately 90% compared to 15–20% (36). The antithrombotic effect of LMW heparins, however, is not related solely to their anti-Xa activity (68–70). Their antithrombotic action may also involve binding to endothelial cells with subsequent release of endogenous compounds (51,53,71,72). LMW heparins have less effect on platelet function than does standard heparin: they react less with thrombin receptors on platelets (67) and generally induce less platelet aggregation, particularly when their mean molecular weight is below 3,000 Da (72). LMW heparins do not increase capillary permeability and microvascular bleeding (54). Some LMW heparins potentiate thrombolysis by tissue plasminogen activator and others do not (52,73). Whereas protamine almost completely neutralizes heparin, it neutralizes different
LMW heparins to varying degrees, ranging from 20-60% (66). Unlike heparin, LMW heparins are primarily excreted by the kidneys (74).

Thus, LMW heparins are theoretically superior to standard heparin: they have a longer half-life, better bioavailability, and should have less bleeding complications due to less effect on platelets and a high anti-Xa:APT ratio. In animal models, these theoretical benefits of LMW heparins hold true (35,36,50).

CLINICAL STUDIES OF LMW HEPARINS

The improved biologic activities and successful animal model studies of LMW heparins have stimulated a number of preliminary clinical studies. Interpretation of these studies has been difficult due to the heterogeneity of LMW heparins and the uncertainty regarding the proper dosage of each agent in humans. Despite certain similar characteristics, different LMW heparins may have very different anticoagulant and antithrombotic properties (36,58,66,72). Since LMW heparins exert their effects by multiple mechanisms, it is as yet unclear to what degree measurements of anti-Xa activity accurately reflect their antithrombotic activity or their hemorrhagic potential. In addition, there is no universal, standardized method of measuring anti-factor Xa levels. Two forms of measurement are commonly used—the amidolytic anti-Xa units (using a chromogenic substrate) and the Institute Choay (IC) anti-Xa units (using a coagulation end point) (35,75). One IC unit equals approximately 0.45 amidolytic anti-Xa units in vitro (75), but direct comparisons between clinical trials remains difficult.

Clinical experience is most extensive in the prophylaxis and treatment of venous thromboembolism. LMW heparins have not proved to be the complication-free drug many had hoped they would be. In an early study, Schmitz-Huebner et al. (76) prematurely discontinued a randomized clinical trial of two subcutaneously administered LMW heparins (LMWH1 and LMWH2) for postoperative DVT prophylaxis due to an unexpected incidence of "severe bleeding." Of 39 patients given heparin, none had DVT or severe bleeding. Of 40 patients given LMWH1, 7% had DVT and 5% had bleeding complications. Of 41 patients given LMWH2, none had DVT, but 22% had severe bleeding complications. It was felt that the longer half-life and increased bioavailability of the LMW heparins may have increased their hemorrhagic risk (75). Subsequent studies emphasized one daily injection, rather than two, for the prophylaxis of venous thromboembolism. Kakkar (77) subcutaneously administered CY 216 (Fraxiparin) once daily to 501 patients for postoperative DVT prophylaxis. Only 3.4% of patients developed DVT and 6.4% developed "excessive" blood loss. The European Fraxiparin Study was a prospective, controlled, multicenter trial comparing subcutaneous CY 216 to subcutaneous unfractionated heparin for postoperative DVT prophylaxis (78). DVT occurred in just 2.8% of 960 patients given CY 216, compared to 4.5% of 936 patients given unfractionated heparin (p < 0.05). Hemorrhagic complications were similar in the two treatment groups.

Bergqvist et al. (69) performed a prospective, randomized, double-blind, multicenter trial of 432 patients comparing subcutaneous Kabi 2165 (Fragmin) to subcutaneous heparin. In an intention-to-treat analysis, DVT occurred in 6.4% of
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the LMW heparin group and in 4.3% of the unfractionated heparin group. Although this difference was not statistically significant, the difference in hemorrhagic complications was (11.6% for Kabi 2165 and 4.5% for heparin). Kakkar et al. (79) compared two regimens of Kabi 2165 for DVT prophylaxis. Of 94 patients given single daily doses, 7.4% developed DVT but only 4.2% had excessive blood loss. Of 112 patients given the same dose twice per day, only 2.6% developed DVT, but 8.9% had excessive blood loss. From these studies, it became clear that single daily injections of Kabi 2165 are effective DVT prophylaxis, but that higher doses induce an increased bleeding tendency (80). Similar conclusions were made following a multicenter trial comparing three single daily dosages of PK 10169 (Enoxaparine) to three injections per day of heparin: the efficacy of DVT prophylaxis was similar for all three doses of the LMW heparin and the unfractionated heparin, and there was a significant bleeding tendency only in those patients receiving the highest dose of PK 10169 (75).

In a small double-blind study (38 patients) on the treatment of established DVT, Holm et al. found that subcutaneous injections twice a day of either Kabi 2165 or standard heparin were equally effective and safe (81). Janvier et al. (82) administered the very LMW heparin CY 222 to 30 patients with established DVT in an open trial. Venographic improvement occurred in 77% of patients and clinical symptoms improved in 93%. This regimen caused no changes in hematologic tests except for increased anti-Xa activity, but, interestingly, the anti-Xa activity was not predictive of the thrombolytic effect. Lockner et al. (83) compared two doses of intravenously administered Kabi 2165 to intravenous heparin in separate, small, randomized trials for the treatment of venographically verified DVT. All patients also received oral warfarin from the first day of treatment. The first study was discontinued prematurely because 2 of 12 patients (16.7%) in the LMW heparin group developed significant postoperative bleeding (both were orthopedic patients). In the second study, a lower dose of intravenous Kabi 2165 was compared to intravenous heparin. Among 29 patients receiving heparin, 48% improved, 41% were unchanged, and 11% progressed on repeat venography. Among 12 patients receiving the higher dose of LMW heparin, 50% improved, 50% were unchanged, and none progressed. Among 13 patients receiving the lower dose of LMW heparin, 77% improved, 23% were unchanged, and none progressed. The incidence of bleeding complications was essentially the same for unfractionated heparin (6.9%) and the lower dose of Kabi 2165 (7.7%). Two case reports describe the successful use of LMW heparins (Kabi 2165 and PK 10169) for the treatment of DVT in pregnant women (84,85).

There have also been clinical trials of LMW heparins in hemodialysis patients, though a difficulty in assessing any new anticoagulant during hemodialysis is that there is no universally accepted optimal dose of heparin (86). Anticoagulation during hemodialysis can be assessed by noting the amount of fibrin deposited on the dialysis membrane; this deposition correlates with the blood levels of fibrinopeptide A (FPA), a small peptide released from fibrinogen after its conversion to fibrin by thrombin (87,88). Ljungberg (87) gave unfractionated heparin to 18 patients during dialysis, and then gave a LMW heparin to the same 18 patients in a subsequent dialysis session. All dialysis sessions were uneventful. FPA levels
were inversely proportional to anti-Xa activity of the anticoagulants. Lane et al. (88) also found that a LMW heparin (Kabi 2165) was as effective as unfractionated heparin for the inhibition of fibrin formation in hemodialysis, and that suppression of FPA levels corresponded well with increased anti-Xa levels. Similar results were found for CY 222 (and the LMW heparinoid, Org 10172), leading Lane and colleagues to conclude that, at least for now, the anti-factor Xa assay is probably the best method of monitoring these antithrombotic agents (86). Borm et al. (89) gave either unfractionated heparin or Kabi 2165 to ten patients during hemodialysis in a single-blind, randomized, crossover study. There were no bleeding complications and the two regimens were equally effective.

LMW heparins are not universally tolerated by patients with heparin-induced thrombocytopenia. In one ex vivo study of platelet-rich plasma from a patient with heparin-induced thrombocytopenia, heparin and several LMW heparins induced irreversible platelet aggregation, while LMW heparinoid and other LMW heparins—including CY 222—did not induce platelet aggregation (90). Another study, however, reported the recurrence of thrombocytopenia in a patient after the administration of CY 222 (91).

Thus, LMW heparins are as effective as conventional unfractionated heparin for the prophylaxis or treatment of DVT and for anticoagulation during hemodialysis. Like unfractionated heparin, however, LMW heparins exhibit dose-dependent hemorrhagic complications. Effective doses of the various LMW heparins have not yet been found that possess less bleeding tendency than equivalent doses of conventional heparin. The heterogeneity of LMW heparins, the uncertainty regarding their proper dosage, the lack of a standardized method of measuring their anti-Xa levels, and the uncertainty of whether or not anti-Xa levels even reflect their antithrombotic and hemorrhagic properties make clinical trials of LMW heparins difficult to interpret. There are no reported clinical trials of LMW heparins for the treatment of ischemic stroke.

**BIOCHEMISTRY, PHARMACOLOGY, AND PREPARATION OF LMW HEPARINOIDS**

Heparinoids are heparin analogues—natural or semisynthetic sulfated glycosaminoglycans (also termed sulfomucopolysaccharides) that are structurally related to heparin and possess certain of its biological properties, such as anticoagulant activity (44,50). These compounds are prepared by tissue extraction or by blending various components (Fig. 1). When originally developed over 30 years ago, they were used as antilipemic-antiatherosclerotic agents (92). Single-component, nonheparin glycosaminoglycans have recently been investigated as antithrombotic agents.

Dermatan sulfate (Fig. 1) is a glycosaminoglycan prepared from tissue extraction with an average molecular weight of 15,000–50,000 Da (93). The only major in vitro activity of dermatan sulfate is its interaction with HC II, which inhibits thrombin (51,94). Dermatan sulfate has no AT III-mediated activity (51). Thus, dermatan sulfate demonstrates weak aPTT activity and little or no inhibition of factor Xa (95). It does affect thrombin time, possibly due to its interaction with

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HC II (51). Dermatan sulfate exhibits only one-half of heparin's in vivo antithrombotic activity in the rabbit stasis model (51). Dermatan sulfate actually decreases capillary permeability and microvascular bleeding and has a protective effect against heparin in this regard (28).

Heparan sulfate is a glycosaminoglycan (Fig. 1) that is similar to heparin in structure, but primarily contains glucuronic acid and 6-sulfated N-acetylgalactosamine residues (96). While it has a lower degree of sulfation than heparin, heparan sulfate is polydisperse with a molecular weight profile similar to that of heparin (51,96). It is prepared from tissue extracts and is a side product in heparin manufacturing. Heparan sulfate may contain a small number of AT III binding sites and thus it weakly potentiates the inhibition of serine proteases—particularly thrombin and factor Xa (60,97-100). The AT III inhibitory effects of heparan sulfate, however, are substantially lower than those of heparin and may be due primarily to the presence of heparin contaminants (46). The antithrombotic activity of heparan sulfate is greater than its anticoagulant activity—it has as much as one-half of heparin's in vivo antithrombotic activity in the rabbit stasis model despite having no significant aPTT or anti-Xa activity. This suggests that the major effect of heparan sulfate is attributable to its interaction with the endothelium (51).

Chondroitin sulfate is structurally similar to deramatan sulfate (Fig. 1), but has no reported anticoagulant or antithrombotic effects. Its presence within certain heparinoid preparations can be ascribed to the inability of manufacturers to remove it from the drug or, possibly, to its presumed effect on the endothelium.

Isolated from porcine intestinal mucosa, Org 10172 is a multicomponent mixture of glycosaminoglycans with mean molecular weight of 6,000 Da that demonstrates physical, chemical, and biological properties associated with each component of the mixture. The composition of Org 10172 was originally described as being 80% heparan sulfate, 10% dermatan sulfate, 5% chondroitin 4- and 6-sulfates, and 5% “heparin-like substance” with high AT III affinity (101,109). The “heparin-like substance” has since been identified as heparan sulfate with high AT III affinity (Magnani H., personal communication). Structurally, Org 10172 is considerably more complex than its individual components. Unlike LMW heparins, Org 10172 contains galactosamine in addition to glucosamine residues and does not contain the altered sugar residues that result from the depolymerization processes (60). Org 10172 has a specific activity of approximately 8 to 10 amidolytic anti-factor Xa U/mg (102,103). It accelerates the rate of inhibition of factor Xa by AT III, but has minimal AT III-mediated inhibitory effect on thrombin (102). Org 10172 differs from LMW heparin in that it contains dermatan sulfate and thus catalyzes inhibition of activated thrombin through HC II. Org 10172 has virtually no effect on platelet function (102,104-106). Org 10172 is an effective antithrombotic agent in rat and rabbit stasis models with less hemorrhagic activity than heparin and several LMW heparins (107,108). It is as yet unclear whether the simple sum or the synergistic effects of Org 10172's components are primarily responsible for its biologic properties.

Because Org 10172 has no appreciable effect on the aPTT, its activity is monitored using plasma inhibition of factor Xa. Anti-Xa levels, however, do not
completely reflect the heparinoid's antithrombotic activity. The differences in the consequences of heparin and Org 10172 on standard laboratory tests of coagulation are summarized in Table 2.

The pharmacokinetics of Org 10172 have been examined in healthy volunteers. By measuring the disappearance of anti-factor Xa activity, subcutaneous or intravenous Org 10172 has an elimination half-life of 19.2 h (101,109). When given intravenously, Org 10172 enters the central compartment \( V_c = 3.9 \) L and then disperses to a total volume of 8.3 L with a distribution half-life of approximately 2.4 h (101,109). These parameters, however, probably reflect only that portion of Org 10172 that exhibits high anti-factor Xa activity and may not describe the disposition of the other components of the compound. Recently, Stieken et al. (109) reviewed the kinetics of the glycosaminoglycans in Org 10172 that do not have a high affinity to AT III. The elimination half-life of these components is rapid (3.5 h) and the plasma clearance is 4.7 L/h. By any method of measurement, the bioavailability of Org 10172 approaches 100% (109). Like heparin, Org 10172 does not appear to necessitate dosage adjustments on body weight. Individual variations in response to Org 10172 may demand dosage adjustments. Because Org 10172 primarily consists of units smaller than 14 saccharides with a high degree of sulfation, its activity on factor Xa is not reversed by protamine (110). Protamine, however, may counteract actions of Org 10172 that are not measured by inhibition of factor Xa. Org 10172 has a 45% renal excretion (111).

Thus, LMW heparinoids—and Org 10172 in particular—may be superior to unfractionated heparin. Org 10172 has better pharmacokinetics with better bioavailability and a much longer half-life. Like LMW heparins, Org 10172 has a high

| TABLE 2. Comparison of ORG 10172 and heparin activity on various hemostatic parameters |
|---------------------------------|------------------|------------------|
| **Parameter**                  | **ORG 10172**    | **Heparin**      |
| General tests                  |                  |                  |
| PT                             |                  |                  |
| aPTT                           | sl   ↑           | ↑                |
| TT                             | sl   ↑           | ↑                |
| Platelet tests                 |                  |                  |
| Bleeding time                  | sl   ↑           | ↑                |
| Aggregation                    | —                | ↑                |
| TXA₂ release                   | —                | ↑                |
| Coagulation assays             |                  |                  |
| Anti-Xa (via AT III)           | ↑    ↑           | ↑                |
| Anti-IIa (via AT III)          | ↑    ↑           | ↑                |
| Anti-IIa (via HC II)           | ↑    ↑           | ↑                |
| Fibrinogen metabolism          | ↓                | ↑                |
| FPA                            | —                | ↑                |
| Fibrinogen                     | sl   ↓           | ↑                |
| Fibrinolysis                   | —                | ↑                |

PT = prothrombin time; aPTT = activated partial thromboplastin time; TT = thrombin time; TXA₂ = thromboxane A₂; AT III = antithrombin III; HC II = heparin cofactor II; FPA = fibrinopeptide A; sl = slight.

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anti-Xa:APTT ratio, yet its effects on platelets are less, and its hemorrhagic tendency in animal models is equal to or less than that of various LMW heparins.

**CLINICAL STUDIES OF ORG 10172**

Org 10172 has been shown to be safe and effective in hemodialysis patients, though with some limitations. Henny et al. (112) used Org 10172 during 55 hemodialysis sessions in 12 patients with acute renal failure and at high risk for bleeding. With plasma anti-Xa levels ranging from 0.42 to 0.93 U/ml (all clinical studies of Org 10172 have utilized the amidolytic assay), no hemorrhagic complications occurred and no fibrin depositions were noted on the dialysis membranes. The same group compared Org 10172 to heparin in a randomized, single-blind, crossover study in 55 patients with chronic renal failure (113). Although fibrin deposition was less during the heparin sessions, the mean anti-Xa levels were higher for heparin and all sessions were without bleeding or clotting complications. In another randomized, crossover study comparing heparin and Org 10172 in chronic renal failure patients, Frei et al. (111) gave each drug to 14 patients for 12 consecutive hemodialysis treatments. Using radioactive iron as a marker, they found no significant difference in blood loss or dialyzer blood retention. Some patients had oozing from the puncture site 24-36 h after the administration of the LMW heparinoid. The mean half-life of anti-Xa activity of Org 10172 was 30.8 h in their patients—nearly double the value found in healthy volunteers. Thus, Org 10172's prolonged half-life and significant renal metabolism may limit its usefulness in patients with renal failure.

A randomized, double-blind, placebo-controlled study in 47 patients undergoing transurethral resection of the prostate (TURP) showed that subcutaneous Org 10172 causes dose-dependent postoperative bleeding (114). In a separate study of 48 TURP patients, three different regimens of subcutaneous Org 10172 caused more postoperative urinary blood loss than did placebo (115).

Org 10172 has been used in a variety of settings for the prevention or treatment of thrombosis. Two randomized, double-blind, placebo-controlled trials of DVT prophylaxis have been performed (103,116). Cade et al. (116) compared three doses of subcutaneous Org 10172 to placebo in 45 patients undergoing major thoracic or abdominal surgery for cancer. DVT occurred in 9 of 14 patients given placebo, 4 of 11 patients given the lowest dose of Org 10172, and none of the 20 patients given the higher doses of heparinoid. Minor injection site hematomas occurred more frequently in patients receiving the heparinoid, but only one patient receiving the highest dose had a significant drop in hemoglobin postoperatively. As in other studies, plasma anti-Xa levels did not correlate with the occurrence of DVT or bleeding, but the levels did show a dose-response relationship (with average midinterval levels of 0.11, 0.18, and 0.26 U/ml). Turpie et al. (103) compared Org 10172 to placebo for DVT prophylaxis in 75 patients with acute (<7 days) atherothrombotic stroke. An intravenous loading dose of Org 10172 was followed by twice-a-day subcutaneous injections. In an intention-to-treat analysis, DVT occurred in 4% of the 50 patients given Org 10172 and in 28% of the 25 given
placebo ($p = 0.005$). A "major" hemorrhage occurred in one patient who suffered hemorrhagic extension of a cerebral infarction after just one injection of Org 10172.

Ten Cate et al. (117) gave intravenous Org 10172 to four patients with intracerebral hematoma and DVT and one patient with a large hemorrhagic infarct and a left ventricular thrombus. One patient died as a result of status epilepticus and had an unexplained drop in hemoglobin level. The other four patients had resolution of their thrombi without bleeding complications. Mean plasma anti-Xa levels for the five patients ranged from 0.63 to 0.96 U/ml. Intravenous Org 10172 has been used successfully to treat at least five patients with heparin-induced thrombocytopenia and thromboembolism (118–120). It was also successfully used for DVT prophylaxis in a pregnant woman with congenital AT III deficiency and a history of type II heparin-induced thrombocytopenia (121). The heparinoid did not cross the placenta. Chong et al. (120) found that Org 10172 cross-reacted with the plasma of 3 of 17 patients (18%) with type II heparin-induced thrombocytopenia, whereas the LMW heparin CY 216 cross-reacted with 16 of 17 (94%) plasma samples.

In the clinical trials of Org 10172 that involved twice daily subcutaneous injections for DVT prophylaxis, no direct correlation existed between plasma anti-Xa levels and antithrombotic or hemorrhagic properties (103,116). Yet there was a dose-dependent increase in anti-Xa levels and patients receiving lower doses were more likely to develop thrombosis while patients receiving higher doses were more likely to develop hemorrhage. The anti-Xa levels did not exceed 0.3 U/ml and trough levels were approximately 75% of midinterval levels. The first attempts to treat patients with constant intravenous infusions and to maintain constant anti-Xa levels were reported in case studies (83,90,118–120). For the treatment of established thrombosis, the "therapeutic" range of plasma anti-Xa levels was felt to be 0.4 to 0.8 U/ml, based on animal experiments (117). In the case reports, these levels were safe and effective and warranted further investigation in clinical trials.

**STUDIES OF ORG 10172 IN PATIENTS WITH ACUTE ISCHEMIC STROKE**

Phase I and phase II trials to evaluate the safety of Org 10172 in patients with acute ischemic stroke have recently been completed (122–124). These studies are also the first in nondialysis patients to aim for constant plasma anti-factor Xa levels via constant intravenous infusion.

In the phase I dose-escalation study, 26 patients were given an intravenous bolus dose followed by a continuous 7-day infusion at an hourly rate of 10% of the bolus (122). One of five different dosing regimens was used (Table 3). No patient required transfusion, treatment for an adverse event, or discontinuation of Org 10172. Only three patients had minor bleeding—epistaxis in a level I patient and heme-positive stools secondary to documented upper gastrointestinal ulcers in patients from level IV and level V. Patients with all subtypes of ischemic stroke were included and, although the purpose of the phase I study was to determine a safe and "optimal" dose, most patients had very favorable outcomes (Table 4).
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**TABLE 3. Dose-escalation schedule of ORG 10172**

<table>
<thead>
<tr>
<th>Level</th>
<th>No. of pts.</th>
<th>ORG 10172 loading dose (anti-Xa U)</th>
<th>ORG 10172 maintenance dose (anti-Xa U/h)</th>
<th>Target plasma level (anti-Xa U/ml)</th>
<th>Actual mean plasma level (anti-Xa U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>625</td>
<td>62.5</td>
<td>0.2</td>
<td>0.16</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1,250</td>
<td>125.0</td>
<td>0.4</td>
<td>0.32</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>1,875</td>
<td>187.5</td>
<td>0.6</td>
<td>0.46</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>2,500</td>
<td>250.0</td>
<td>0.8</td>
<td>0.63</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>3,125</td>
<td>312.5</td>
<td>1.0</td>
<td>0.72</td>
</tr>
</tbody>
</table>

From ref. 122.

The 3-month mortality was zero and almost 50% of patients had complete recovery. Outcome and bleeding tendency were not dose dependent.

Several patients required adjustment of their infusions. Plasma anti-Xa levels reached desired levels after the loading dose, but immediately thereafter decreased to a nadir at 6 h. The levels then gradually increased to a plateau at day 4. Thus, Org 10172 exhibited a multicompartimental kinetic profile. The regimen was changed in the phase II trial: a more rapid maintenance infusion was used initially in order to avoid a postbolus nadir in anti-Xa levels (124). In the phase I trial, three level V patients achieved anti-Xa levels greater than 1.0 U/ml. Since this level was considered too high, the phase II trial utilized a modification of level IV in order to maintain plasma anti-factor Xa levels of 0.8 U/ml (124).

The phase II trial of 57 patients was therefore designed to evaluate the safety of Org 10172 at high doses and to evaluate a new dosing regimen for the maintenance of steady-state anti-Xa levels (124). Following a loading dose of 2,500 U, a three-stage infusion was used: 600 U/h for 4 h, 400 U/h for 12 h, and then 300 U/h for the remainder of the 7-day course. Although many patients still required adjustment of dosages, there was no postbolus nadir of anti-Xa level and the daily mean anti-Xa levels remained relatively constant in a range between 0.5 and 0.8 U/ml.

Minor hemorrhagic side effects not requiring discontinuation of heparinoid oc-

**TABLE 4. Neurologic conditions of patients given ORG 10172 for acute ischemic stroke**

<table>
<thead>
<tr>
<th></th>
<th>24 h after treatment, N (%)</th>
<th>7 days after treatment, N (%)</th>
<th>3 months after treatment, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (26 patients)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>14 (53.8)</td>
<td>20 (76.9)</td>
<td>25 (96.2)</td>
</tr>
<tr>
<td>Unchanged</td>
<td>8 (30.8)</td>
<td>2 (7.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Worse</td>
<td>4 (15.4)</td>
<td>4 (15.4)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Dead</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Phase II (57 patients)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>27 (47.4)</td>
<td>41 (71.9)</td>
<td>40 (71.4)%</td>
</tr>
<tr>
<td>Unchanged</td>
<td>21 (36.8)</td>
<td>9 (15.8)</td>
<td>8 (14.3)%</td>
</tr>
<tr>
<td>Worse</td>
<td>9 (15.8)</td>
<td>5 (8.8)</td>
<td>2 (3.6)%</td>
</tr>
<tr>
<td>Dead</td>
<td>0 (0.0)</td>
<td>2 (3.5)</td>
<td>6 (10.7)%</td>
</tr>
</tbody>
</table>

* From ref. 122.

* From ref. 124.

* Based on 56 patients after one patient lost to follow-up.
curred in four patients: two with epistaxis and two with heme-positive stools. Major hemorrhagic side effects occurred in three patients. One patient with gastrointestinal hemorrhage, hematuria, and a decrease in hematocrit recovered without requiring transfusion. Two elderly patients died after suffering hemorrhagic transformations of large, cardioembolic, hemispheric infarctions. The first had an anti-Xa level of 1.22 U/ml and a mean blood pressure of 140 torr when the event occurred 6 h after initiating treatment. The other patient had mass effect on the admission computed tomography (CT) scan, though the plasma anti-Xa level was only 0.6 U/ml when the event occurred on day 6. After the two fatalities, patients with cerebral edema and midline shift on initial CT scan and those with mean arterial blood pressure greater than 130 torr were excluded from the trial.

In the phase II study, outcomes were generally favorable and quite similar to those observed in the phase I study (Table 4). The 15.8% rate of neurologic worsening at 24 h was better than the 28.2% rate reported by Duke et al. (3) for nontreated patients. The 5.3% occurrence of major hemorrhage compared favorably to results from heparin trials. At 3 months, 65% of patients in the phase II trial had no or minimal disability. The 3-month mortality rate of 10.7% was lower than the range of 14 to 28% reported in various other natural history studies and clinical trials (125–130). In addition, only the two deaths secondary to intracranial hemorrhage occurred during the acute treatment period. The other causes of death were recurrent ischemic stroke in two patients, pulmonary embolism in one, and pneumonia and renal failure in one. The fatal recurrent strokes occurred 8 and 10 weeks after the original events. The pulmonary embolism also occurred several weeks following the stroke. A multicenter, randomized, placebo-controlled trial testing the utility of Org 10172 in the management of patients with acute ischemic stroke is now underway.

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