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Recombinant Coagulation Factors and Thrombolytic Agents

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INTRODUCTION

Coagulation and fibrinogenolysis exist in a mutually compensatory or balanced state. Endogenous regulatory mechanisms ensure that the processes of hemostasis and blood coagulation at a site of injury, and the subsequent fibrinolysis of the blood clot, are localized and well regulated. This ensures a rapid and efficient hemostatic response at a site of injury while avoiding thrombogenic events at sites distant from the site of injury or the hemostatic response from persisting beyond its physiologic need. This chapter will focus on recombinant products that are available to facilitate coagulation and for thrombolysis.

Two models of blood hemostasis, a cascade model and a cell-based model, have been proposed. A schematic of the cascade and cell-based models of coagulation is presented in Fig. 15.1.

The initial model of coagulation was proposed in the 1960s, encompassing a series of steps, or cascade, where enzymes cleaved a zymogen to generate the subsequent enzyme. In the cascade model, coagulation was divided into the intrinsic and extrinsic pathways. The extrinsic pathway was located outside the blood and consisted of tissue factor-dependent cofactors and enzymes and factor VIIa. The intrinsic system was localized within the blood and could be initiated through contact activation of factor XII, which leads to activation of subsequent components. The two pathways converged into the common pathway, leading to the generation of thrombin. While the cascade model was useful in explaining how the coagulation enzymatic steps occurred in vitro and in helping interpret laboratory tests, it did not adequately explain the hemostatic process as it occurs in vivo.

More recently, a cell-based model of coagulation has been proposed (Hoffman 2003; Hoffman and Monroe 2005). This cell-based model emphasizes the interaction of clotting factors with cell surfaces and appears to explain some of the unresolved issues with the cascade model. The cell-based model of coagulation comprises four phases: initiation, amplification, propagation, and termination. The initiation phase is localized to cells expressing tissue factor (TF), which are generally localized outside the vasculature. Upon injury, blood is exposed to cells bearing TF, and factor VIIa rapidly binds to exposed TF. The TF-VIIa complex activates additional factor VII to factor VIIa which in turn activates small amounts of factor IX and factor X. Factor Xa binds factor Va to form the prothrombinase complex, which cleaves prothrombin to thrombin. During amplification, the small amount of thrombin generated diffuses away from the TF-bearing cells and activated platelets, exposing receptors and binding sites for activated clotting factors. Once platelets are activated, the release of granule contents leads to recruitment of additional platelets to the site of injury, leading to the propagation phase on the surface of activated platelets. The propagation step culminates in a burst of thrombin generation of sufficient magnitude to clot fibrinogen that is converted to fibrin. Once the fibrin platelet clot has formed over the site of injury, the clotting process must be terminated to prevent thrombotic occlusion.

Normally hemostasis is a highly efficient and tightly regulated process to ensure that it occurs quickly and is localized. Abnormalities that result in a delay in blood coagulation are associated with a bleeding tendency termed hemophilia. Hemophilia is an X-linked recessive disorder that affects approximately 400,000 people worldwide (Shapiro et al. 2005). Hemophilia A (classical hemophilia) patients have decreased, defective, or absent production of factor

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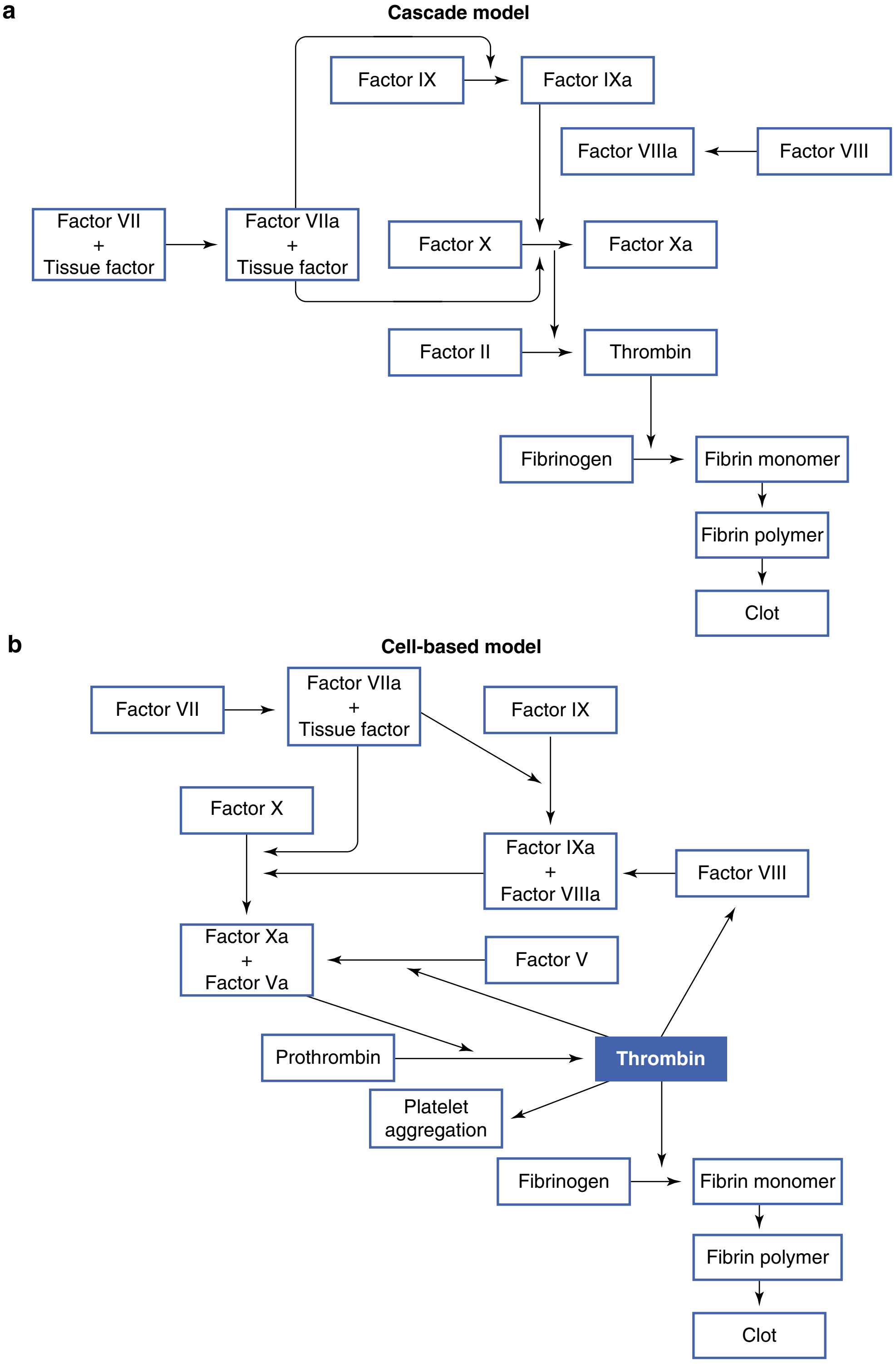

Figure 15.1 ■ Schematic representation of fibrinolytic pathway. Panel **a**: Cascade model; Panel **b**: Cell-based model.

VIII. It affects approximately 1 in 5,000–10,000 males. Patients with hemophilia B lack factor IX, affecting 1 in 20,000–30,000 males. Factor XI deficiency, originally termed hemophilia C, an autosomal recessive disorder, is less common (affecting 1 in 100,000 patients in the American population) and in most cases is a mild bleeding disorder. The availability of recombinant coagulation factors has been a major advance in the treatment of hemophilia, providing the promise of unlimited supply, ease of use, improved safety, and reducing the risk of infections transmitted by transfusion.

FACTOR VIII

Factor VIII (antihemophilia factor) is a plasma protein that functions as a cofactor by increasing the maximum catabolic capacity ($V_{\max}$) in the activation of factor X by factor IXa in the presence of calcium ions and negatively charged phospholipid. The congenital absence of factor VIII is termed hemophilia A and afflicts approximately 1 in 5,000–10,000 males.

■ Structure

Factor VIII is synthesized as a single-chain polypeptide of 2,332 amino acids. Shortly after synthesis, cleavage occurs, and most plasma factor VIII circulates as an 80-kD light chain (A3-C1-C2 domains) associated with a series of 90–210-kD heavy chains (A1-A2-B domains) in a metal ion-dependent complex. There are 25 potential N-linked glycosylation sites and 22 cysteines (Vehar et al. 1984a).

■ Pharmacology

The concentration of factor VIII in plasma is about 200 ng/mL (Hoyer 1981). It is not known where factor VIII is synthesized although evidence suggests that several different tissues, including the spleen, liver, and kidney, may play a role. Factor VIII is normally covalently associated with a 50-fold excess of von Willebrand factor. Von Willebrand factor protects factor VIII from proteolytic cleavage and allows concentration at sites of hemostasis. Circulating von Willebrand factor is bound by exposed subendothelium and activated platelets at sites of injury, which allows localization of von Willebrand factor and factor VIII.

Factor VIII circulates in the body as a large precursor polypeptide devoid of coagulant activity. Cleavage by thrombin at Arg372-Ser373, Arg740-Ser741, and Arg1689-Ser1690 results in its procoagulant structure (Vehar et al. 1984a). While cleavage at Arg740 is not essential for coagulant activity, cleavage at the other two sites is necessary. Although factor VIII is synthesized as a single-chain polypeptide, the single-chain polypeptide is cleaved shortly after synthesis. Most of the factor VIII in plasma exists as an 80-kD light chain and a series of heavy chains. Factor VIII circulates as a heterodimer of the 80-kD light chain and a variable (90–210 kD) heavy chain in a metal ion-dependent complex. Small acidic regions in the C-terminal portion of A1 and A2 and in the N-terminal part of A3 are required for optimal coagulation activity, while the B-domain is not directly necessary for procoagulant activity.

RECOMBINANT FACTOR VIII

Recombinant factor VIII (rFVII) is available from three sources: Baxter, Bayer, and Pfizer. Recombinant factor VIII products may be divided into three classes based on the use of human or mammalian-derived raw materials (Table 15.1). Recombinant factor VIII from Baxter (Advate®, Recombinate®) and Pfizer (ReFacto®, Xyntha®) is produced using transfected Chinese hamster ovary (CHO) cells, whereas that from Bayer (Kogenate®, Kogenate FS, Helixate® FS) is produced using transfected baby hamster kidney cells. A major difference between rFVIII from Bayer and Baxter is the presence of a Galα1 → 3Gal carbohydrate moiety in the Baxter product (Hironaka et al. 1992). The recombinant product from Baxter and Bayer consists of full-length factor VIII which, like plasma-derived factor VIII, consists of a dimer of the 80-kD light chain and a heterogeneous heavy chain of 90- to 210-kD (Schwartz et al. 1990). The Pfizer products (ReFacto® and Xyntha®) are a deletion mutant in which the heavy chain lacks nearly the entire B-domain, which is not needed for clotting activity (Roddie and Ludlam 1997). After proteolytic cleavage by thrombin, the activated B-domain-depleted molecule is essentially identical to the activated full-length native rFVIII.

■ Pharmaceutical Considerations

Advate® recombinant antihemophilic factor-Plasma/Albumin Free Method (rAHF-PFM, Baxter) is formulated as a sterile, non-pyrogenic, lyophilized cake for intravenous injection and is provided in single-dose vials, containing nominally 250, 500, 1,000, or 1,500 international units (IU). Biological potency is determined using an in vitro assay that employs a factor VIII concentrate standard that is referenced to the World Health Organization (WHO) International Standard for factor VIII:C concentrates. The specific activity is 4,000–10,000 IU/mg of protein. The final product contains no preservative nor added human or animal components in the formulation. Recombinant antihemophilic factor is administered only by intravenous infusion following reconstitution with 5 mL sterile water for injection. The product contains mannitol, trehalose, sodium, histidine, Tris, calcium, polysorbate 80, and glutathione. Plastic syringes must be used since the protein can adhere to glass syringes.

Recombinant factor VIII (Kogenate®, Bayer; Recombinate®, Baxter; ReFacto®, Xyntha®, Pfizer) is supplied as sterile, single-dose vials containing 250–3,000 IU of factor VIII. The preparation is lyophilized and stabilized with human albumin (Kogenate® and Recombinate®) or polysorbate 80 (ReFacto®, Xyntha®).

Product (manufacturer)	Viral inactivation procedure(s)	Available strengths (IU)
First Generation (media enriched with human or animal plasma proteins)		
Recombinate® (Baxter Healthcare)	Immunoaffinity, ion-exchange chromatography	250, 500, 1,000
Human albumin as stabilizer	Bovine serum albumin used in culture medium for CHO cells	
Second Generation (human albumin-free final formulations)		
Kogenate FS® (Bayer Healthcare)	Immunoaffinity chromatography	250, 500, 1,000, 2,000
Helixate® FS (Bayer for CSL Behring)	Ion exchange	
Sucrose as stabilizer	Solvent detergent (TNBP/polysorbate 80)	
	Ultrafiltration	
ReFacto® (Wyeth) B-domain deleted	Ion exchange	250, 500, 1,000, 2,000
Sucrose as stabilizer	Solvent detergent (TNBP/Triton X-100)	
(Not available after 2009)	Nanofiltration	
Third Generation (no human or animal protein used in culture medium or manufacturing process; does contain trace amounts of murine monoclonal antibody)		
Advate® (Baxter Healthcare)	Immunoaffinity chromatography	250, 500, 1,000, 1,500, 2,000, 3,000
Trehalose as stabilizer	Ion exchange	
	Solvent detergent (TNBP/polysorbate 80)	
Xyntha® (Pfizer) B-domain deleted	Nanofiltration	250, 500, 1,000, 2,000
Sucrose as stabilizer	Solvent detergent	
	Polysorbate 80	

Table 15.1 ■ Summary of recombinant factor VIII concentrates.

Recently a reformulated product, Kogenate® FS (Bayer), has become available. This product is similar to its predecessor Kogenate antihemophilic factor but incorporates a revised purification and formulation process that eliminates the addition of human albumin as a stabilizer, instead of using histidine. The products contain no preservatives and should be stored at 2–8 °C. The lyophilized powder may be stored at room temperature (up to 25 °C) for up to 3 months without loss of biological activity. Freezing should be avoided. Factor VIII should be reconstituted with the diluent provided. The reconstituted product must be administered intravenously by direct syringe injection or drip infusion within 3 h of reconstitution.

■ Clinical Usage

Recombinant factor VIII (Kogenate® and Kogenate® FS, Bayer Corporation; Recombinate® and Advate®, Baxter; ReFacto®, Xyntha®, Pfizer) is indicated for the control and prevention of bleeding episodes in adults and children (0–16 years) with hemophilia A, the perioperative management in adults and children with hemophilia A, and the routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adults and children with hemophilia A. Recombinant factor VIII is not indicated for the treatment of von Willebrand disease.

The pharmacokinetics of rFVIII is summarized in Table 15.2. The increase in factor VIII concentration is dose proportional and the disposition is similar following single and chronic dosing.

Dosage of rFVIII (in IU) must be individualized to the needs of the patient, the severity of the deficiency and of the hemorrhage, the presence of inhibitors, and to the desired increase in factor VIII activity (in IU/dL, or percentage of normal). The required dosage of factor VIII may be estimated as follows:

$$\text{Required dose}\,(\text{IU}) = \text{Body weight}\,(\text{kg}) \times \text{desired rise in factor VIII}\left(\frac{\text{IU}}{\text{dL}} \text{ or \% of normal}\right) \times 0.5 \left(\frac{\text{IU}}{\text{kg}} \text{ per } \frac{\text{IU}}{\text{dL}}\right)$$

	Dose (IU/kg)	Population	N	C_{max} (IU/dL)	CL (mL/h/kg)	V_{ss} (mL/kg)	MRT (h)	$t_{1/2}$ (h)	Reference
Plasma and albumin-free (rAHF-PFM, Baxter Bioscience)	50	Previously treated pediatric patients <6 years of age	47[a]	95.6 ± 23.3	4.3 ± 1.4	51.4 ± 12.9	12.5 ± 3.1	9.9 ± 1.9	Blanchette et al. (2008)
Plasma and albumin-free (rAHF-PFM, Advate®, Baxter Bioscience)	50	Pediatric, 1–6 years age	52	92	4.3	50	NR	9.4	Björkman et al. (2010)
	50	Previously treated patients, 10–65 years	100	122	3.3	48	NR	11.2	
rFVIII (Kogenate FS®, Bayer)	50	Children (<18 years age)	20	NR	4.1	59.2	15.1	10.7	Barnes et al. (2006)
BDD-rFVIII, (ReFacto®, Pfizer)	25	Patients (>12 years age) with severe hemophilia	21	NR	3.0 ± 1.0	62.7 ± 18.7[b]	24.3 ± 17	17.7 ± 13	Morfini et al. (2003)

Abbreviations: *AUC* area under the curve, C_{max} maximum concentration, *CL* clearance, *MRT* mean residence time, V_{dss} volume of distribution, $t_{1/2}$ half-life, *NR* not reported

[a] Per protocol population

[b] V_{darea}

Table 15.2 ■ Clinical pharmacokinetic profile of recombinant factor VIII.

Local manufacturer's prescribing information should be consulted for full details of indications, dosage regimens, administration, precautions and warning, and regional differences in prescribing.

Safety

Trace amounts of mouse or hamster protein may be present in certain rFVIII as contaminants from the manufacturing process. Therefore, caution should be exercised when administering rFVIII to individuals with known hypersensitivity to plasma-derived antihemophilic factor or with hypersensitivity to biological preparations with trace amounts of murine or hamster proteins.

FACTOR VIIA

The development of recombinant factor VIIa was motivated by the fact that a small fraction of patients with hemophilia (15–20 % of patients with hemophilia A and 2–5 % of patients with hemophilia B) develop antibodies (inhibitors) to factor VIII and factor IX, respectively. High titers of inhibitors make it impossible to give sufficient coagulation factor to overcome the inhibitor, and therapy is ineffective or is associated with unacceptable side effects. Factor VIIa plays a central role in coagulation according to the cell-based concept of coagulation. In the absence of tissue factor, factor VII has very low proteolytic activity whereas factor VIIa requires either tissue factor or activated platelets (tissue factor independent) to generate thrombin. Hereditary deficiency in factor VII is rare, affecting one symptomatic individual per 500,000 population (Lapecorella and Mariani 2008).

■ Structure

Factor VII is a vitamin K-dependent glycosylated serine protease proenzyme that is synthesized in the liver. It has 406 amino acids and the molecular weight is ~50 kDa. The protein is not functionally active unless it is γ-carboxylated. There are two sites of N-linked glycosylation on factor VII. Factor VII is synthesized in the liver as a proenzyme that becomes activated and cleaved upon hydrolysis at Arg-152 and Ile-153. The two polypeptide chains comprise a light (152 residues) and heavy (254 residues) chain. The light chain contains an N-terminal γ-carboxyglutamic acid (Gla) domain, in which all 10 Gla residues are posttranslationally γ-carboxylated followed by two domains homologous to epidermal growth factor (EGF). The heavy chain comprises a serine protease domain which is homologous to trypsin.

■ Pharmacology

The concentration of factor VII in plasma is approximately 0.5 mcg/mL. The factor VII zymogen forms a high-affinity complex with cell-bound tissue factor. Activated factor VII (FVIIa) cleaves factor X and factor IX, eventually leading to the formation of thrombin.

RECOMBINANT FACTOR VIIA

Recombinant factor VII is expressed in baby hamster kidney cells as a single-chain form and is spontaneously autoactivated to factor VIIa during purification with almost 100 % yield (Thim et al. 1988). The purification of rFVII from the cell culture medium involves pH adjustment of the medium and loading onto a Q-Sepharose FF column to concentrate the protein, treatment with a detergent to ensure virus inactivation, loading onto an immunoaffinity column to purify rFVII using a monoclonal antibody that recognizes the FVII Gla domain in its functional conformation, final purification, and complete activation of rFVII to rFVIIa through the use of two anion-exchange chromatography steps (Jurlander et al. 2001). The resulting FVIIa is then formulated, dispensed into vials, and freeze dried. The characterization of the protein indicates that it is very similar to plasma-derived factor VIIa with regard to amino acid sequence. In plasma-derived FVIIa, all 10 possible Gla residues are fully γ-carboxylated, whereas rVIIa contains 9 fully and 1 partially (50 %) γ-carboxylated residue (Thim et al. 1988). Recombinant FVIIa is approved for use in hemophilia with inhibitors and in patients with acquired hemophilia in Europe, the United States, and Japan.

■ Pharmacokinetics and Pharmacodynamics

The single-dose pharmacokinetics of recombinant factor VIIa was first investigated in 15 patients with hemophilia with severe factor VIII or factor IX deficiency (Lindley et al. 1994). Following an intravenous dose of 17.5, 35, and 70 μg/kg, the plasma clearance was 30.3, 32.4, and 36.1 mL/h/kg, respectively. The pharmacokinetics was linear, and no difference in clearance was noted between nonbleeding and bleeding episodes. Median clearance was 31.0 mL/h in nonbleeding episodes and 32.6 mL/h in bleeding episodes. The median half-life was 2.9 h in nonbleeding episodes and 2.3 h in bleeding episodes. Table 15.3 summarizes the pharmacokinetics of rFVIIa in various populations. In patients with hemophilia or factor VII deficiency, the clearance, volume of distribution, elimination half-life, and mean residence time appear independent of dose. Plasma clearance appears similar in healthy adults and patients with hemophilia whereas clearance is generally faster in children than in adults. There are no significant gender differences. The pharmacokinetics of rFVIIa appears similar in healthy Caucasian and Japanese subjects (Table 15.4). In a summary of the pharmacokinetics, Klitgaard and Nielsen categorized subjects falling in two groups in terms of rVIIa pharmacokinetics: in healthy volunteers, adult patients with hemophilia, and nonbleeding patients with cirrhosis, plasma clearance is low (30–40 mL/h/kg), whereas in children with hemophilia, patients with congenital FVII deficiency, and patients with active, high levels of bleeding, plasma clearance appears higher (60–90 mL/h/kg) (Klitgaard and Nielsen 2007).

In the absence of FVIII or FIX, doses of rFVIIa greater than 25 nM are required to induce hemostasis. Although the concentrations of rFVIIa for maximum prolongation of clot lysis time varies widely, the median concentration was 73.0 U/mL (Lisman et al. 2002), roughly equivalent to a rFVIIa dose of 90–120 μg/kg (Hedner 2006; 2007).

■ Pharmaceutical Considerations

NovoSeven® (Novo Nordisk) is supplied as a white lyophilized powder in single-use glass vials formulated with sodium chloride, calcium chloride dihydrate, glycylglycine, polysorbate 80, and mannitol. The pH is adjusted to 5.3–6.3. The product does not contain any stabilizing protein. Before reconstitution, NovoSeven® should be stored refrigerated (2–8 °C) avoiding exposure to direct sunlight. NovoSeven® is distributed in vials of 1.2 mg (60,000 IU), 2.4 mg (120,000 IU), or 4.8 mg (240,000 IU) to be reconstituted with sterile water for injection. After reconstitution with the appropriate volume of diluent, each vial contains approximately 0.6 mg/mL. Following reconstitution, NovoSeven® may be stored refrigerated or at room temperature for up to 3 h. NovoSeven® is intended for intravenous bolus injection and should not be mixed with infusion solutions.

A formulation of rFVIIa that is stable at room temperature (rFVII-RT, NovoSeven® RT, Novo Nordisk) is formulated with sodium chloride, calcium chloride dihydrate, glycylglycine, polysorbate 80, mannitol, sucrose, and methionine. The formulation was shown to be bioequivalent to rFVIIa based on data from a crossover study comparing the two formulations at 90 μg/kg (Bysted et al. 2007). NovoSeven® RT is distributed in vials of 1 mg (50,000 IU), 2 mg (100,000 IU), 5 mg (250,000 IU), or 8 mg (400,000 IU) with a diluent comprising 10 mmol solution of L-histidine in water for injection.

Dose (mcg/kg)	Population	*N*	C_{max} (U/mL)	CL (mL/h/kg)	V_{ss} (mL/kg)	MRT (h)	$t_{1/2}$ (h)	Reference
<20	Healthy volunteers anticoagulated with acenocoumarol		NR	30.9 ± 5.1	80 ± 8	NR	2.4 ± 0.2	Girard et al. (1998)
>20	Healthy volunteers anticoagulated with acenocoumarol		NR	34.5 ± 7.0	94 ± 15	NR	2.5 ± 0.3	Girard et al. (1998)
90 rFVIIa	Healthy male volunteers	22	55.4 ± 7.9	37.6 ± 6.0	111 ± 18	3.0 ± 0.3	3.5 ± 0.3	Bysted et al. (2007)
90 rfVIIa-RT	Healthy male volunteers	22	52.8 ± 7.3	40.4 ± 6.2	123 ± 20	3.1 ± 0.3	3.5 ± 0.3	Bysted et al. (2007)
17.5–70	Adult hemophiliac, bleeding	5–21	9.9 ± 6.3	36.6 ± 8.7	104 ± 25	2.7 ± 0.5	2.5 ± 0.5	Lindley et al. (1994)
17.5–70	Adult hemophiliac, nonbleeding	25–29	11.4 ± 7.7	32.1 ± 12.2	110 ± 39	3.5 ± 0.6	2.8 ± 0.5	Lindley et al. (1994)
15	Patients with severe congenital deficiency in factor VII	5	NR	64.9 ± 22.7	210 ± 70	3.3 ± 0.5	2.4 ± 0.4	Berrettini et al. (2001)
30	Patients with severe congenital deficiency in factor VII	5	NR	67.7 ± 17.9	230 ± 70	3.5 ± 0.6	2.6 ± 0.6	Berrettini et al. (2001)
90–180	Children with hemophilia A	12	NR	78	196	2.5	2.3	Villar et al. (2004)
90	Adults with hemophilia A	5	NR	53	159	3.0	2.3	Villar et al. (2004)
17.5–70	Adults with hemophilia A or B with or without inhibitors	15	10.8 ± 7.1	32.8 ± 11.7	108.9 ± 37.2	3.3 ± 0.6	2.7 ± 0.5	Lindley et al. (1994)
100–200	Trauma subjects 16–65 years of age	230	NR	40	120	NR	2.4	Klitgaard et al. (2006)
5	Adult nonbleeding patients with cirrhosis and prolonged PTT (>2 s above upper limit of reference range)	10	2.3 ± 0.6	32.9 ± 16.9	NR	3.1 ± 1.3	2.4	Bernstein et al. (1997)
20		10	6.1 ± 3.3	43.7 ± 20.0	NR	4.0 ± 2.3	3.2	Bernstein et al. (1997)
80		10	30.8 ± 6.1	34.9 ± 16.5	NR	3.1 ± 0.5	2.9	Bernstein et al. (1997)

Abbreviations: *AUC* area under the curve, C_{max} maximum concentration, *CL* clearance, *MRT* mean residence time, V_{dss} volume of distribution, $t_{1/2}$ half-life, *NR* not reported

Table 15.3 ■ Clinical pharmacokinetics of recombinant factor VIIa.

Dose (mcg/kg)	Population	*N*	C_{max} (U/mL)	CL (mL/h/kg)	V_{ss} (mL/kg)	$t_{1/2}$ (h)	Reference
40	Caucasian	11	13.4 ± 2.3	34.3 ± 4.9	145 ± 37	5.2 ± 2.7	Fridberg et al. (2005)
	Japanese	11	12.1 ± 1.4	33.3 ± 5.0	165 ± 42	6.0 ± 2.4	
80	Caucasian	12	24.1 ± 1.9	36.6 ± 5.2	132 ± 13	3.9 ± 1.1	
	Japanese	11	25.2 ± 2.4	33.7 ± 3.0	130 ± 18	4.3 ± 0.9	
160	Caucasian	11	45.9 ± 6.3	37.2 ± 6.3	139 ± 22	4.1 ± 1.0	
	Japanese	10	48.0 ± 5.9	34.5 ± 4.6	135 ± 14	4.0 ± 0.6	

C_{max} maximum plasma concentration, *CL* plasma clearance, V_1 initial volume of distribution, V_{ss} steady-state volume of distribution, $t_{1/2}$ half-life

Table 15.4 ■ Comparison of recombinant-activated FVII pharmacokinetics in Caucasians and Japanese.

Clinical Usage

Recombinant factor VIIa (NovoSeven® and NovoSeven® RT) is indicated for the treatment of bleeding episodes in patients with congenital hemophilia A (deficiency of factor VIII) or B (deficiency of factor IX) with inhibitors. It is also indicated for use in patients who are congenitally deficient in factor VII and for prophylaxis of surgical bleeding in patients with hemophilia A or B and in patients who have acquired hemophilia with inhibitors to factor VIII or IX. In the European Union and in Japan, recombinant factor VIIa is also indicated for the control of Glanzmann's thrombasthenia with antibodies to platelet membrane glycoprotein IIb/IIIa and/or human leukocyte antigen and with past or present refractoriness to platelet transfusion.

The recommended dose of recombinant factor VIIa for patients with hemophilia A or B with inhibitors is 90 μg/kg every 2 h by bolus infusion until hemostasis is achieved or until treatment is judged to be inadequate. The dose for Glanzmann's thrombasthenia approved in the European Union is 90 μg/kg every 2 h for a minimum of 3 doses. In the EU a single-dose injection of 270 μg/kg was recently approved. For patients with factor VII deficiency, the initial dosage is 15–30 μg/kg every 4–6 h until hemostasis is achieved. Local manufacturer's prescribing information should be consulted for full details of indications, dosage regimens, administration, precautions and warning, and regional differences in prescribing.

Safety

The main safety concern with rFVIIa is the risk of thromboembolic adverse events because rFVIIa is administered in supraphysiological (~1,000-fold) doses. A review of safety data up to April 2003, involving more than 700,000 administrations of 90 μg/kg, indicates a low level of serious adverse events (1 %) including thrombotic events such as myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis, and disseminated intravascular coagulation (Abshire and Kenet 2004). In an updated review covering the period from May 2003 to December 2006, with approximately 800,000 doses of rFVIIa (90 μg/kg), there were a total of 30 thromboembolic events and 6 thromboembolic event-associated fatal events (Abshire and Kenet 2008).

Recombinant factor VIIa should not be administered to patients with known hypersensitivity to recombinant factor VIIa or any of the components of recombinant factor VIIa. Recombinant factor VIIa is contraindicated in patients with known hypersensitivity to mouse, hamster, or bovine proteins.

Recent Developments

Recombinant VII is rapidly cleared with a short terminal half-life (2.4 h), requiring multiple, frequent administrations (2–3 doses given at 2- or 3-h intervals). Various approaches, including rFVII variants with site-directed amino acid substitution, conjugation with polyethylene glycol or carrier protein such as albumin or Fc component of IgG, and incorporation into pegylated liposomes, have been explored to prolong the residence time.

NN1731, a variant of rFVIIa with three amino acid substitutions (V158D, E296V, M298Q) designed to stabilize the molecule in the active conformation without tissue factor has been evaluated in healthy subjects (Møss et al. 2009).

A recent report indicated the development of a recombinant FVIIa-albumin fusion protein (Schulte 2008). In vitro characterization demonstrated that the specific molar activity of the rVIIa-albumin fusion protein was 70 % of that of wild-type rFVIIa. A pharmacokinetic study in rats showed that the rVIIa-albumin fusion protein had a half-life that was 5.8 times longer than NovoSeven®. An in vivo FVII depletion model in rats involving treatment with phenprocoumon showed that the rFVIIa-albumin fusion corrected the coagulation time comparable to NovoSeven® and showed a longer duration of effect.

Several groups have evaluated the use of liposomes (cf. Chap. 4) to deliver FVIIa. Recent studies have shown that rFVII formulated with pegylated liposomes improved the hemostatic efficacy in vitro (Yatuv et al. 2008), an animal model (Yatuv et al. 2010), and more recently in phase I/II clinical study (Spira et al. 2010).

FACTOR IX

Factor IX is activated by factor VII/tissue factor complex in the extrinsic pathway and by factor XIa in the intrinsic pathway (Fig. 15.1). Activated factor IX, in combination with activated factor VIII, activates factor X, resulting in the conversion of prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin, forming a blood clot at a site of hemorrhage.

RECOMBINANT COAGULATION FACTOR IX

Recombinant factor IX is a 415 amino acid glycoprotein with a molecular weight of ~55 kD. Recombinant coagulation factor IX (BeneFIX®, Pfizer) is produced in a CHO cell line. The transfected cell line secretes recombinant factor IX in the culture medium from which the protein is purified via several steps (Monahan and Di Paola 2010). The condition medium undergoes ultrafiltration/diafiltration to concentrate the protein and establish a consistent buffer. This is followed by four sequential column chromatography steps. The first chromatographic step involves binding to Q-Sepharose Fast Flow resin and subsequent elution with calcium chloride. The second step is running over Cellufine Sulfate, a heparin analog, to achieve further affinity purification. The third step involves running over a column consisting of a synthetic form of calcium phosphate in macroporous particles to retain factor IX based on charge and removes lower activity forms of rFIX. The final column involves immobilized copper to retain factor IX and remove trace host cell and other contaminants. The purified rFIX is subsequently passed through an ultrafiltration membrane and undergoes final diafiltration/ultrafiltration to concentrate and exchange the rFIX to the final formulation buffer.

While plasma-derived factor IX carries a Thr/Ala dimorphism at position 148, the primary amino acid sequence of recombinant factor IX is identical to the Ala148 allelic form. As a result of posttranslational modifications, recombinant and plasma-derived factor IX differ in a number of respects (White et al. 1997). First, plasma-derived factor IX carries 12 gamma-carboxyglutamic acid (Gla) residues in its amino-terminal Gla-domain, whereas 40 % of recombinant factor IX is undercarboxylated, lacking gamma-carboxylation at Glu40. Other differences between recombinant and plasma-derived factor IX are in the activation peptide region (residues 146–180), which is cleaved off upon factor IX activation. These include the lack of sulfation at Tyr155 and of phosphorylation at Ser158, as well as different N-linked glycosylation patterns at Asn157 and Asn167.

The potency of recombinant factor IX is determined in international units using an in vitro clotting assay. One international unit is the amount of factor IX activity present in a milliliter of pooled normal human plasma. The specific activity of BeneFIX® is greater than or equal to 200 IU/mg protein.

■ Pharmacology

Pharmacokinetic and pharmacodynamic studies have indicated that increases in recombinant factor IX plasma concentrations are correlated with factor IX activity. Comparison of recombinant factor IX and plasma-derived factor IX in a dog model of hemophilia B indicated that while plasma-derived factor IX had a higher AUC and C_{max} compared with recombinant factor IX, the efficacy of the two products was similar (Keith et al. 1995).

■ Pharmaceutical Considerations

In 2007, a reformulation of recombinant factor IX (BeneFIX®) was implemented, replacing the original formulation. The reformulation increased the ionic strength but still retaining iso-osmolality, allowed a more concentrated preparation, and replaced sterile water for reconstitution with 0.234 % sodium chloride. Additional improvements included a needleless reconstitution device, a prefilled diluent syringe, and a similar dilution volume for all dosage strengths. BeneFIX® is supplied as a sterile, non-pyrogenic, lyophilized powder in single-use vials containing nominally 250, 500, 1,000, 2,000, or 3,000 IU per vial.

The product labeled for room temperature storage may be stored at room temperature (not to exceed 30 °C) or under refrigeration (2–8 °C). The product labeled for refrigerated storage should be stored at 2–8 °C. Prior to the expiration date, the product may be stored at room temperature, not to exceed 30 °C for up to 6 months. Freezing should be avoided to prevent damage to the diluent vial.

Recombinant factor IX is administered by intravenous infusion after reconstitution of the lyophilized powder with the supplied prefilled diluent (0.234 % sodium chloride solution) syringe. The reconstituted solution may be stored at room temperature prior to administration, but BeneFIX® should be administered within 3 h of reconstitution since it does not contain a preservative. Upon reconstitution, BeneFIX® contains polysorbate 80 which is known to increase the rate of di-(2-ethylhexyl)phthalate extraction from polyvinyl chloride. This should be considered during preparation and administration of BeneFIX®, including any storage following reconstitution.

After reconstitution, BeneFIX® should be injected intravenously over several minutes. BeneFIX® should be administered using the tubing provided in the kit. In addition, the solution should be withdrawn from the vial using the vial adapter provided. Reconstituted BeneFIX® should not be administered in the same tubing or container with other medicinal products.

■ Clinical Usage

BeneFIX® is indicated for the control and prevention of bleeding episodes in adults and pediatric patients with hemophilia B (congenital factor IX deficiency or Christmas disease) and for perioperative management in adult and pediatric patients with hemophilia B.

BeneFIX® is contraindicated in patients who have manifested life-threatening, immediate hypersensitivity reactions, including anaphylaxis, to the product or its components, including hamster protein.

The in vivo recovery using BeneFIX® was 25–30 % less than the recovery using highly purified plasma-derived factor IX, whereas there was no difference in the biological half-life (White et al. 1997). The pharmacokinetics of recombinant factor IX is summarized in Table 15.5. Several reports in previously untreated hemophilia B patients and patients previously treated with plasma-derived FIX indicate a very high rate of efficacy (>80 %) with rFIX.

The dosage and duration of substitution treatment depend on the severity of factor IX deficiency, the location and extent of bleeding, the clinical condition, patient age, and the desired recovery in factor IX. The initial estimated dose may be determined as follows:

$$\begin{aligned}&\text{Required units} = \text{Body weight}\left(\text{kg}\right)\\&\quad\times\text{desired factor IX increase}\left(\text{IU / dL or \% of normal}\right)\\&\quad\times\text{reciprocal of observed recovery}\left(\text{IU / kg per IU / dL}\right)\end{aligned}$$

In clinical studies, one IU of BeneFIX® per kilogram of body weight (average recovery) increased circulating factor IX as follows:

Adults: 0.8 ± 0.2 IU/dL (range 0.4–1.2)
Pediatrics (<15 years): 0.7 ± 0.3 IU/dL (range 0.2–2.1)

Higher doses of factor IX may be necessary in patients with inhibitors. If the expected levels of factor IX are not attained, or if bleeding is not controlled, biological testing may be merited to determine if factor IX inhibitors are present.

Local manufacturer's prescribing information should be consulted for full details of indications, dosage regimens, administration, precautions and warning, and regional differences in prescribing.

Safety

Since BeneFIX® is produced in a CHO cell line, it is contraindicated in patients with known history of hypersensitivity to hamster protein and other constituents in the preparation. During uncontrolled open-label clinical studies with recombinant factor IX, adverse events reported in more than 2 % of patients included nausea, taste perversion, injection site reaction, injection site pain, headache, dizziness, allergic rhinitis, rash, hives, flushing, fever, and shaking.

■ Recent Developments

When treating bleeding episodes, several infusions of factor IX may be required to maintain sufficient coagulation factor levels. Recombinant factor IX products with a longer half-life may provide less frequent dosing, potentially improving compliance.

N9-GP is a modified recombinant serum-free factor IX obtained through site-directed glycopegylation wherein a 40 kDA polyethylene glycol is attached to the FIX activation peptide. Upon activation, the activation peptide, including the attached PEG, is cleaved off releasing the active FIX. A recent pharmacokinetic study in patients with hemophilia B and a FIX activity ≤2 % showed that N9-GP had a half-life that

Age (years)	*N*	IVR (IU/dL per U/kg)	CL (mL/h/kg)	V_{ss} (mL/kg)	MRT (h)	$t_{1/2}$ (h)	Reference
4–9	11	0.61 ± 0.2	10.4 ± 2.3	270 ± 70	26 ± 4.9	20 ± 4.2	Björkman et al. (2001)
10–19	10	0.79 ± 0.3	8.3 ± 2.3	210 ± 70	25 ± 5.3	20 ± 4.1	
20–29	12	0.67 ± 0.2	8.5 ± 1.2	220 ± 60	26 ± 6.2	19 ± 4.9	
30–39	12	0.84 ± 0.2	7.2 ± 1,4	190 ± 40	27 ± 7.7	20 ± 6.5	
40–49	7	0.80 ± 0.3	7.6 ± 1,7	200 ± 50	27 ± 4.7	19 ± 4.2	
50–56	3	0.88 ± 0.2	7.5 ± 0.3	180 ± 80	24 ± 9.6	17 ± 7.1	

Table 15.5 ■ Clinical pharmacokinetic profile of recombinant factor IX.

was approximately five times longer than the patient's previous FIX product and the AUC was approximately eightfold higher compared to pdFIX and tenfold higher compared to rFIX (Negrier et al. 2011).

Recombinant factor IX-Fc fusion protein (rFIXFc) comprises a single molecule of recombinant FIX attached to the constant region of IgG. The presence of the Fc domain allows binding to the neonatal Fc receptor, protecting the Fc-containing molecular from catabolism. In mice, rats, monkeys, and FIX-deficient mice and dogs, rFIXFc was shown to have a three- to four-fold extended half-life compared to rFIX (Peters et al. 2010). In FIX-deficient mice, rFIXFc and rFIX at comparable doses resulted in a similar correction of the clotting deficiency, but rFIXFc had a prolonged effect to 96 h. These results were extended to humans in a phase I/2a open-label study in previously treated patients with hemophilia B (Shapiro et al. 2011). A dose proportional increase in C_{max} was noted, and the elimination half-life was ~54–58 h for rFIXFc compared to the reported half-life of 19 h for BeneFIX®.

FACTOR XIII

Factor XIII is a plasma transglutaminase and the terminal enzyme in the clotting cascade, increasing clot strength by cross-linking fibrin, and increases fibrinolytic resistance by incorporating α_2-plasmin into the clot matrix. Congenital factor XIII deficiency is a rare autosomal recessive disorder affecting one in five million persons (Di Paola et al. 2001).

RECOMBINANT COAGULATION FACTOR XIII

Recombinant factor XIII is an investigational product produced in yeast *Saccharomyces cerevisiae* as a nonglycosylated FXIII A_2 homodimer which is equivalent to cellular FXIII normally found in platelets and readily forms the heterotetrameric A_2B_2 complex in the presence of free FXIII B subunits. Recombinant factor XIII is captured by concentration of the fermentation broth, homogenization of cells, and purification by several chromatography steps.

■ Clinical Usage

The pharmacokinetics of recombinant factor XIII has been studied in healthy volunteers (Reynolds et al. 2005; Visich et al. 2005) and patients with congenital factor XIII deficiency (Lovejoy et al. 2006). Following a single intravenous injection of 50 U/kg rFXIII in healthy volunteers, the estimated half-life was 270–320 h, the volume of distribution ranged from 40 to 75 mL/kg, and FXIII activity increased by 1.77 % per 1 U/kg rFXIII administered (Reynolds et al. 2005). In a multiple-dose study investigating doses of 10 and 25 U/kg in healthy volunteers, the elimination half-life ranged from 228 to 346 h for the 10 U/kg dose and 167 to 197 h for the 25 U/kg dose, and a three- to four-fold accumulation of rFXIII was noted following five daily doses (Visich et al. 2005). In a phase I escalating-dose study in patients with factor XIII deficiency, rFXIII-A_2 complexed with endogenous FXIII B subunits with a half-life of 8.5 days, similar to endogenous FXIII. The median dose response was a 2.4 % increase in FXIII activity per U/kg rFXIII administered (Lovejoy et al. 2006).

RECOMBINANT THROMBOLYTIC AGENTS

■ Tissue-Type Plasminogen Activator

Deposition of fibrin and platelets in the vasculature leads to thromboembolic diseases that are responsible for considerable mortality and morbidity. Early thrombolytic therapy can decrease mortality and improve coronary-artery patency in patients with acute myocardial infarction (AMI). During fibrinolysis, the inactive zymogen plasminogen is enzymatically converted to the active moiety, plasmin, which in turn digests the insoluble fibrin matrix of a thrombus to soluble fibrin degradation products. Tissue-type plasminogen activator (t-PA) exhibits fibrin-specific plasminogen activation with minimal systemic fibrinogenolysis. The relative absence of systemic fibrinogenolysis with t-PA means that there are fewer systemic side effects compared to other plasminogen activators. Mean t-PA antigen concentrations at rest in humans are approximately 5 μg/mL and can increase 1.5- to 2-fold in venous occlusion (Holvoet et al. 1987).

■ Structure

Native t-PA is a serine protease synthesized by vascular endothelial cells as a single-chain polypeptide of 527 amino acids with a molecular mass of 64 kD (Pennica et al. 1983). Approximately 6–8 % of the molecular mass consists of carbohydrate. A schematic of the primary structure of human t-PA is shown in Fig. 15.2. There are 17 disulfide bridges and an additional free cysteine at position 83 and 4 putative N-linked glycosylation sites recognized by the consensus sequence Asn-X-Ser/Thr at residues 117, 184, 218, and 448 (Pennica et al. 1983). In addition, the presence of a fucose attached to Thr61 via an O-glycosidic linkage has been reported (Harris et al. 1991). Two forms of t-PA that differ by the absence or presence of a carbohydrate at Asp184 have been characterized: Type I t-PA is glycosylated at asparagine 117, 184, and 448; whereas Type II t-PA lacks a glycosylation at asparagine 184. The asparagine at amino acid 218 is normally not occupied in either form of t-PA (Vehar et al. 1984b). Asparagine 117 contains a high-mannose

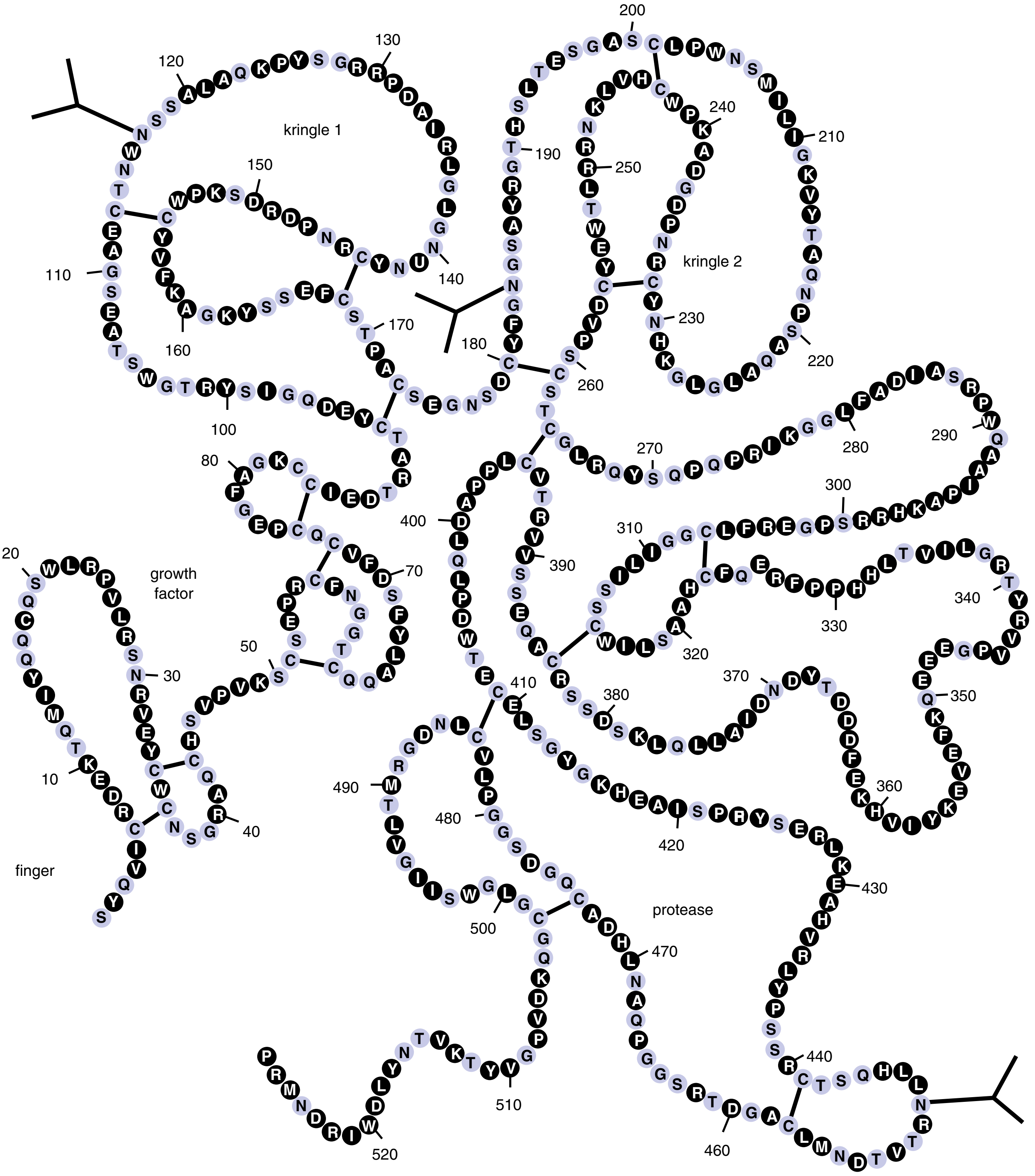

Figure 15.2 ■ Primary structure of tissue-plasminogen activator.

oligosaccharide whereas Asn184 and Asn448 are of the complex carbohydrate type (Spellman et al. 1989). Complex N-linked glycan structures contain a disaccharide Galβ(1,4)GlcNac and terminate in sialic acid residues, while an oligomannose (high mannose)-type glycan contains only mannose in the outer arms.

During fibrinolysis, the single-chain t-PA polypeptide is cleaved between Arg275 and Ile276 by

Administration regimen	Health status	C_{max} (µg/mL)	CL (L/min)	V_1 (L)	V_{ss} (L)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	$t_{1/2}\gamma$ (h)	Reference
0.25 mg/kg/30 min	Healthy	0.96 ± 0.18	0.64 ± 0.05	4.6 ± 0.3	8.1 ± 0.8	4.4 ± 0.2	39 ± 2.6	–	Tanswell et al. (1989)
0.5 mg/kg/30 min	Healthy	1.8 ± 0.25							
100 mg/2.5 h	AMI	3.3 ± 0.95	0.38 ± 0.07	2.8 ± 0.9	9.3 ± 5.0	3.6 ± 0.9	15 ± 5.4	3.7 ± 1.4	Seifried et al. (1989)
100 mg/1.5 h	AMI	4 ± 1	0.57 ± 0.1	3.4 ± 1.5	8.4 ± 5	3.4 ± 1.4	72 ± 68	–	Tanswell et al. (1992)
100 mg/1.5 h	AMI	–	0.45 ± 0.17	7.2 ± 4	28.9 ± 22	–	144 ± 100	–	Modi et al. (2000)
100 mg/1.5 h	AMI		0.39	6.7	17.3	7.4	22.3	228	Kostis et al. (2002)
Bolus	AMI	9.8 ± 3.6	0.48 ± 0.15	4.5 ± 1.3	31 ± 18	4.8 ± 1.0	17 ± 6.3	9.1 ± 3.1	Tebbe et al. (1989)

C_{max} maximum plasma concentration, *CL* plasma clearance, V_1 initial volume of distribution, V_{ss} steady-state volume of distribution, $t_{1/2}$ half-life

Table 15.6 ■ Pharmacokinetic parameters (mean ± SD) for alteplase antigen following intravenous administration in healthy volunteers and patients with AMI.

plasmin to yield 2-chain t-PA. Two-chain t-PA consists of a heavy chain (A-chain) derived from the amino terminus and a light chain (B-chain) linked by a single disulfide bridge between Cys264 and Cys395. The A-chain consists of the finger, growth factor, and 2 kringle domains. The finger domain and the second kringle are responsible for t-PA binding to fibrin and for the activation of plasminogen. The function of the first kringle is not known. The B-chain contains the serine protease domain consisting of the His-Asp-Ser triad that cleaves plasminogen (Pennica et al. 1983).

FIRST-GENERATION RECOMBINANT THROMBOLYTIC AGENTS: RECOMBINANT T-PA (RT-PA)

Recombinant t-PA (rt-PA) (alteplase) is identical to endogenous human t-PA. Like melanoma-derived t-PA, rt-PA lacks glycosylation at Asn218 and exists in two forms that differ by the absence or presence of a carbohydrate at residue Asn184. Type II t-PA has a slightly higher specific activity in vitro compared with Type I t-PA.

■ Pharmacokinetics of rt-PA

The pharmacokinetics of rt-PA has been studied in mice, rats, rabbits, primates, and humans. After intravenous administration, the plasma concentrations decline rapidly with an initial dominant half-life of less than 5 min in all species. Plasma clearance ranges from 27 mL/min in rabbits (Hotchkiss et al. 1988) to 620 mL/min in humans (Tanswell et al. 1989). Recombinant t-PA exhibits nonlinear (Michaelis-Menten) pharmacokinetics at high plasma concentrations (Tanswell et al. 1990). The estimated Michaelis-Menten constant (K_m) and V_{max} values estimated by simultaneously fitting multiple plasma concentration-time curves following several doses were 12–15 µg/mL and 3.7 µg/mL/h, respectively, with little species variation in these parameters. The pharmacokinetics is essentially linear in cases where plasma concentrations do not exceed 10–20 % of K_m (i.e., 1.5–3 µg/mL). A pharmacokinetic summary of alteplase following intravenous administration in humans is presented in Table 15.6. These data show that rt-PA has an initial volume of distribution approximating plasma volume and a rapid plasma clearance. The initial half-life was less than 5 min. There was no difference in the pharmacokinetics following the different infusion regimens. A lower plasma clearance was noted following intravenous bolus injection, suggesting saturation of clearance mechanisms.

The primary route of alteplase clearance is via receptor-mediated clearance mechanisms in the liver. Three cell types in the liver are responsible for the clearance of t-PA: parenchymal cells, endothelial cells, and Kupffer cells. Kupffer cells and endothelial cells mediate t-PA clearance via the mannose receptor. Parenchymal cells clear t-PA via a carbohydrate-independent, receptor-mediated mechanism. Data suggest that this carbohydrate-independent clearance is mediated by the low-density lipoprotein receptor-related protein (LRP) (Bu et al. 1993).

■ Pharmaceutical Considerations

Recombinant human t-PA (Alteplase; Activase®, Genentech, Inc; Actilyse®, Boehringer Ingelheim) is supplied as a sterile, white to off-white lyophilized powder in 50 mg vials containing vacuum and 100 mg vials without vacuum. Cathflo Activase is supplied as a sterile, lyophilized powder in 2 mg vials. Recombinant t-PA is practically insoluble in water, and arginine is included in the formulation to increase aqueous solubility. Phosphoric acid and/or sodium hydroxide may be used to adjust the pH. The sterile lyophilized powder should be stored at controlled room temperatures not to exceed 30 °C or refrigerated at 2–8 °C, and it should be protected from excessive light.

The powder is reconstituted by aseptically adding the accompanying sterile water for injection to the vial, resulting in a colorless to pale yellow transparent solution containing 1 mg/mL rt-PA, with a pH of approximately 7.3 and an osmolality of approximately 215 mOs/kg. Recombinant t-PA is stable in solution over a pH range of 5–7.5. Since the reconstituted solution does not contain any preservatives, it should be used within 8 h of preparation and should be stored at 2–30 °C. The solution is incompatible with bacteriostatic water for injection. Other solutions such as sterile water for injection or preservative-containing solutions should not be used for further dilution. The 1 mg/mL solution can be diluted further with an equal volume of 0.9 % sodium chloride for injection, USP or 5 % dextrose injection USP to yield a solution with a concentration of 0.5 mg/mL. This solution is compatible with glass bottles and polyvinyl chloride bags.

■ Clinical Usage

Recombinant human t-PA (alteplase t-PA) is indicated for use in the management of acute myocardial infarct (AMI) in adults for the improvement of ventricular function following AMI, reduction of the incidence of congestive heart failure, reduction of mortality associated with AMI, and for the management of acute massive pulmonary embolism in adults. It is also indicated for the management of acute ischemic stroke in adults for improving neurological recovery and reducing the incidence of disability if therapy is initiated within 3 h after the onset of stroke symptoms and after exclusion of intracranial hemorrhage by cranial computerized tomography (CT) scan or other diagnostic imaging method sensitive for the presence of hemorrhage.

Alteplase (Cathflo® Activase®) is indicated for the restoration of function to central venous access devices as assessed by the ability to withdraw blood.

Two dose regimens, the 90-min accelerated regimen and the 3-h regimen, have been studied in patients experiencing AMI; controlled studies comparing the clinical outcome of the two regimens have not been conducted. For the accelerated regimen, the recommended dose is based on patient weight, not to exceed 100 mg alteplase. For patients weighing more than 67 kg, the recommended dose regimen is 100 mg as a 15-mg intravenous bolus injection, followed by 50 mg infused over 30 min and then 35 mg infused over the next 60 min. For patients weighing no more than 67 kg, the recommended dose regimen is a 15-mg intravenous bolus injection, followed by 0.75 mg/kg infused over 30 min not to exceed 50 mg and then 0.5 mg/kg over the next 60 min not to exceed 35 mg.

For the 3-h regimen, the recommended dose is 100 mg administered as 60 mg in the first hour (6–10 mg as a bolus) and 20 mg over each of the second and third hours. For patients weighing less than 65 kg, the dose is 1.25 mg/kg over 3 h. Infarct artery-related patency rates of 70–77 % are achieved at 90 min with this 3-h regimen (Verstraete et al. 1985). Patency grades of blood flow in the infarct-related artery are defined by the Thrombolysis in Myocardial Infarction (TIMI) scale and are assessed angiographically with TIMI grade 0 representing no flow; grade 1, minimal flow; grade 2, sluggish flow; and grade 3, complete or full, brisk flow.

The efficacy of the accelerated 90-min regimen was demonstrated in an international, multicenter trial, Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO), that enrolled approximately 41,000 patients (The GUSTO Investigators 1993a, b). The GUSTO trial demonstrated a higher infarct-related artery patency rate at 90 min in the group treated with rt-PA with heparin compared with streptokinase with either intravenous or subcutaneous heparin. The patency in the alteplase group was 81.3 % compared with 53.5–59.0 % in the streptokinase groups. In addition, the alteplase group had a reduced mortality (an additional 10 lives saved per 10,000 patients treated). The intracranial hemorrhage rate was approximately 1 %.

In a multicenter, open-label study in 461 patients with AMI randomized to receive 100 mg alteplase over 90 min or two 50-mg bolus doses 30 min apart, the 90-min angiographic patency rate was 74.5 % for the double-bolus group and 81.4 % in the infusion group ($p = 0.08$). The 30-day mortality rates were 4.5 % in the bolus group and 1.7 % in the infusion group (not significantly different) (Bleich et al. 1998). Similarly, the Continuous Infusion Versus Double-Bolus Administration of Alteplase (COBALT) trial in 7,169 patients with AMI showed a higher incidence of 30-day mortality in the double-bolus alteplase group (7.98 %) compared with the accelerated-infusion group (7.53 %). There was also a slightly higher incidence of intracranial hemorrhage in the double-bolus group (COBALT Investigators 1997).

For acute ischemic stroke, the recommended dose is 0.9 mg/kg not to exceed 90 mg infused over 60 min, with 10 % of the total dose administered as an initial intravenous bolus over 1 min. The safety and efficacy of this regimen with concomitant use of heparin and aspirin during the first 24 h has not been investigated.

The recommended dose for treatment of pulmonary embolism is 100 mg administered by intravenous infusion over 2 h. Heparin therapy should be instituted or reinstituted near the end of or immediately following alteplase infusion when the partial thromboplastin time or thrombin time returns to twice normal or less.

Safety Concerns

Since thrombolytic therapy increases the risk of bleeding, alteplase is contraindicated in patients with a history of cerebrovascular accidents or patients who have any kind of active internal bleeding, intracranial neoplasm, arteriovenous malformation, or aneurism or who have had recent intracranial or intraspinal surgery or trauma.

SECOND-GENERATION RECOMBINANT THROMBOLYTIC AGENTS

The rapid clearance of rt-PA from the circulation by the liver necessitates administration as an intravenous infusion. Although alteplase provides more rapid thrombolysis and superior patency compared with streptokinase and urokinase at therapeutic doses, there is some fibrinogenolysis and the administration scheme is relatively complicated. Thus, there is room for further improvements in efficacy and safety. Considerable nonclinical and clinical research has been underway to identify rt-PA variants that are fibrin specific and that have a simpler administration regimen compared with alteplase. Strategies that have been used to develop t-PA variants have included domain deletions, glycosylation changes, or site-directed amino acid substitutions. A number of these second-generation thrombolytic agents are currently in late-stage clinical trials or have been approved for marketing.

■ Reteplase

Reteplase is a 355-amino deletion variant of t-PA, consisting of the protease and kringle 2 domains of human t-PA. It is expressed in *Escherichia coli* cells as a single-chain, nonglycosylated, 39.6-kDa peptide.

■ Pharmacology

Like alteplase, reteplase is a fibrin-specific activator of plasminogen. In vitro, the plasminogenolytic activity of reteplase is 2- to 3.8-fold lower than alteplase on a molar basis (Kohnert et al. 1993), which may be attributed to the absence of the finger domain in reteplase. Reteplase had a similar in vitro maximal efficacy ($E_{\max}$) compared with alteplase. However, the molar concentration required to produce 50 % clot lysis (EC_{50}) was 6.4-fold higher for reteplase than for alteplase (Martin et al. 1993). The data also suggested that in vitro, reteplase has a lower thrombolytic potency in lysing aged and platelet-rich clots compared with alteplase.

Due to the deletion of the finger region, epidermal growth factor domain, and kringle 1, as well as the carbohydrate side chains, the hepatic clearance of reteplase is reduced. A summary of the pharmacokinetics of reteplase in humans is presented in Table 15.7.

■ Pharmaceutical Considerations

Reteplase is supplied as a sterile, white, lyophilized powder for intravenous injection after reconstitution with "sterile water for injection, USP" supplied as part of the kit. Following reconstitution, the pH of the solution is 6.0. Reteplase contains no antibacterial preservatives and should be reconstituted immediately before use. The solution should be used within 4 h when stored at 2–30 °C.

■ Clinical Usage

Reteplase (Retavase®, Rapilysin®) is indicated for use in the management of AMI in adults for the improvement of ventricular function following AMI, the reduction of the incidence of congestive heart failure and the reduction of mortality associated with AMI.

The potency of reteplase is expressed in units using a reference standard that is specific for reteplase and is not comparable with units used for other thrombolytic agents. Reteplase is administered as a double-bolus injection regimen consisting of 10 U each. Each bolus is administered as an intravenous bolus injection over 2 min via an intravenous line in which no other medications are being administered simultaneously. The second bolus injection is given 30 min after the first. Heparin and reteplase are incompatible when combined in solution and should not be administered simultaneously through the same intravenous line. If reteplase is administered through an intravenous line containing heparin, normal saline or 5 % dextrose solution should be flushed through the intravenous line before and following reteplase.

The International Joint Efficacy Comparison of Thrombolytics (INJECT) trial evaluated the effects of reteplase (10 + 10 U) and streptokinase (1.6 million Units over 60 min) on 35-day mortality in 6,010 AMI patients in a double-blind randomized fashion. The 35-day mortality was 9.0 % for patients treated with reteplase and 9.5 % for those treated with streptokinase with no difference between the two groups. The incidence of stroke was also similar between the groups.

Dose	Population	*N*	C_{max}	CL (L/h)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (h)	Reference
Reteplase[a]							
10 U	AMI patients	4	4,620	6.24	19.2	6.3	Seifried et al. (1992)
15 U	AMI patients	9	5,060	8.34	18.8	6.3	Seifried et al. (1992)
15 U	AMI patients	9	5,170	8.70	21.4	5.0	Grünewald et al. (1997)
10 U + 5 U	AMI patients	7	3,610	9.12	16.3	5.4	Grünewald et al. (1997)
10 U + 10 U	AMI patients	8	3,370	6.90	17.0	5.5	Grünewald et al. (1997)
Tenecteplase[b]							
30 mg	AMI patients	48	10.0	98.5	21.5	1.93	Modi et al. (2000)
40 mg	AMI patients	31	10.9	119	23.8	2.15	Modi et al. (2000)
50 mg	AMI patients	20	15.2	99.9	20.1	1.50	Modi et al. (2000)
Lanoteplase							
15 kU/kg	AMI patients	4	NR	3.42	36	12	Kostis et al. (2002)
30 kU/kg	AMI patients	2	NR	3.12	31	7.4	Kostis et al. (2002)
60 kU/kg	AMI patients	8	NR	3.42	32	8.7	Kostis et al. (2002)
120 kU/kg	AMI patients	8	NR	2.4	47	10.5	Kostis et al. (2002)
10 kU/kg	Healthy young male	9	NR	2.6	32	9.4	Vachharajani et al. (2011)
10 kU/kg	Healthy young female	10	NR	2.8	30	8.7	Vachharajani et al. (2011)
10 kU/kg	Healthy elderly male	10	NR	3.0	28	8.5	Vachharajani et al. (2011)
10 kU/kg	Healthy elderly female	10	NR	2.0	31	9.8	Vachharajani et al. (2011)

Abbreviations: *AUC* area under the curve, C_{max} maximum concentration, *CL* clearance, *MRT* mean residence time, V_{dss} volume of distribution, $t_{1/2}$ half-life, *NR* not reported

C_{max} values are reported in [a]U/mL or [b]ng/mL

Table 15.7 ■ Pharmacokinetic parameters for second-generation recombinant thrombolytic agents.

However, more patients treated with reteplase experienced hemorrhagic strokes.

Two open-label angiographic studies (Reteplase Angiographic Phase II International Dose-finding study [RAPID 1] and Reteplase versus Alteplase Patency Investigation During myocardial infarction [RAPID 2]) have compared reteplase with alteplase. In RAPID 1 patients were treated with reteplase (10 + 10 U, 15 U, or 10 + 5 U) or the standard alteplase regimen (100 mg over 3 h) within 6 h of symptoms. Ninety-minute TIMI grade 3 flow was seen in 63 % of patients in the 10 + 10 U reteplase group and 49 % of the patients in the standard regimen alteplase group.

RAPID 2 was an open-label, randomized trial in 320 patients comparing 10 + 10 U reteplase and accelerated alteplase within 12 h of symptom onset. Percentages of patients with TIMI grade 3 flow at 90 min were 59.9 % in the reteplase group and 45.2 % in the alteplase group. There was no significant difference in the 35-day mortality between the two groups. Neither trial was powered to compare the efficacy or safety with respect to mortality or incidence of stroke.

The more favorable results for reteplase compared with alteplase noted in smaller trials were not replicated in a large, randomized, double-blind trial. In the GUSTO III trial, 15,059 patients were randomized in a 2:1 fashion to receive reteplase in 2 bolus doses of 10 U 30 min apart or up to 100 mg alteplase infused over 90 min. The 30-day mortality rates were 7.47 % for reteplase and 7.24 % for alteplase (The Global Use of Strategies to Open Occluded Coronary Arteries [GUSTO III] Investigators 1997). The stroke rate was 1.64 % for reteplase and 1.79 % for alteplase ($p = 0.50$). Reteplase, while easier to administer than accelerated alteplase, did not demonstrate any survival advantage.

Safety Concerns

As with other thrombolytic agents, reteplase is contraindicated in cases of active internal bleeding, history of cerebrovascular accident, recent intracranial or intraspinal surgery or trauma, intracranial neoplasm, arteriovenous malformation, or aneurism, in cases of known bleeding diathesis, or in severe uncontrolled hypertension.

■ Tenecteplase

Tenecteplase (TNKase®, Genentech, Inc; Metalyse®, Boehringer Ingelheim) is a t-PA variant that has amino acid substitutions in three regions of t-PA. Replacement of threonine at amino acid 103 by asparagine (T103N) incorporates a complex oligosaccharide carbohydrate structure at this position. The replacement of arginine at position 117 by glutamine (N117Q) results in the removal of the high-mannose carbohydrate present at this site. A tetra-alanine substitution at positions 496–499 (KHRR496–499AAAA) contributes to increased fibrin specificity. These three design modifications result in a thrombolytic that, compared to the parent t-PA molecule, is approximately 10- to 14-fold more fibrin specific, is 80-fold more resistant to local inactivation, and has an 8-fold slower clearance in rabbits (Keyt et al. 1994).

■ Pharmacology

Like alteplase, tenecteplase has Type I and Type II glycoforms. Type I has 3 carbohydrate structures at asparagine 103, 184, and 448; and Type II lacks the carbohydrate at asparagine 184. Carbohydrate structures on tenecteplase are all of the complex oligosaccharide type with no high-mannose structures. For this reason, the rapid mannose receptor-mediated clearance observed for alteplase does not occur with tenecteplase. Rather, tenecteplase is thought to be cleared by galactose receptors present in liver sinusoidal cells.

Enzymatic removal of terminal sialic acid from tenecteplase has been shown to increase the clearance in rabbits and is likely due to increased exposure of underlying galactose sugars. This desialylation effect is more profound with tenecteplase than with alteplase and is probably due to the predominant mannose-receptor-mediated clearance for alteplase. A second possible clearance pathway for tenecteplase is a noncarbohydrate-mediated mechanism via the low-density lipoprotein receptor-related protein (LRP) that is also a clearance pathway for alteplase (Camani et al. 1998).

The thrombolytic potency of tenecteplase was five- to tenfold greater than alteplase in animal models of coronary-artery thrombosis (Benedict et al. 1995) and embolic stroke (Thomas et al. 1994). The slower clearance of tenecteplase results in a longer exposure of the clot to the thrombolytic agent, which likely offsets the slightly lower activity. The higher fibrin specificity of tenecteplase results in lower systemic activation of plasminogen and an observed conservation of fibrinogen.

■ Pharmaceutical Considerations

Tenecteplase is supplied as a sterile, white to off-white, lyophilized powder in a 40-mg (Metalyse®) or 50-mg vial (Metalyse®, TNKase®) under a partial vacuum. Each vial is packaged with sterile water for injection for reconstitution and syringe with a dual cannula device. It should be stored at controlled room temperature not to exceed 30 °C, or it should be stored refrigerated at 2–8 °C. Tenecteplase is intended for intravenous bolus injection following reconstitution with sterile water for injection. Each vial nominally contains a 5 % overfill, L-arginine, phosphoric acid, and polysorbate 20. The biological potency of tenecteplase is determined by an in vitro clot lysis assay and is expressed in tenecteplase-specific activity units. The specific activity of tenecteplase has been defined as 200 units/mg protein.

■ Clinical Usage

Tenecteplase is indicated for the reduction of mortality associated with AMI. The recommended total dose of tenecteplase should not exceed 50 mg and is based on patient weight according to the following weight-adjusted dosing table (Wang-Clow et al. 2001):

Patient weight (kg)	Tenecteplase dose (mg)
<60	30
≥60 to <70	35
≥70 to <80	40
≥80 to <90	45
≥90	50

Treatment should be initiated as soon as possible after the onset of AMI symptoms. Tenecteplase is contraindicated in patients with known bleeding diathesis or active internal bleeding, history of cerebrovascular accident, recent intracranial or intraspinal surgery or trauma, intracranial neoplasm, arteriovenous malformation, aneurysm, or severe uncontrolled hypertension due to an increased risk of bleeding.

The clinical pharmacokinetics of tenecteplase have been examined in two studies (TIMI 10A and TIMI 10B). TIMI 10A was a phase 1 pilot safety study in patients with AMI. Pharmacokinetic data were obtained in 82 patients following intravenous bolus doses of 5–50 mg. Tenecteplase plasma concentrations decreased in a biphasic manner with an initial half-life of 11–20 min and a terminal half-life of 41–138 min. Mean plasma clearance of tenecteplase ranged from 125 to 216 mL/min and decreased with increasing dose (Modi et al. 1998).

TIMI 10B was a dose-finding phase 2 efficacy study comparing 30-, 40-, and 50-mg doses of bolus tenecteplase to 100 mg alteplase administered via the accelerated-infusion regimen. The pharmacokinetic data from TIMI 10B are summarized in Table 15.7. Tenecteplase plasma clearance was approximately

100 mL/min compared to 453 mL/min for accelerated alteplase. In contrast to TIMI 10A, no dose-dependent decrease in plasma clearance was noted in TIMI 10B, likely as a result of the narrower dose range examined. Additionally, the plasma clearances noted in TIMI 10B were slightly lower than those noted in TIMI 10A at comparable doses (Modi et al. 2000). The 30-, 40-, and 50-mg doses in TIMI 10B produced TIMI grade 3 flow in 54.3, 62.8, and 65.8 % of the patients, respectively. TIMI grade 3 flow was seen in 62.7 % of patients in the accelerated alteplase group, not significantly different from that in the 40-mg tenecteplase group. An additional finding of this dose-finding efficacy trial was that dose-adjusted dosing is important in achieving optimal reperfusion (Cannon et al. 1998). In addition, tenecteplase resulted in a lower change from baseline in systemic coagulation factors compared with alteplase.

The safety and efficacy of tenecteplase were studied in a large double-blind, randomized trial (Assessment of the Safety and Efficacy of a New Thrombolytic [ASSENT-2]). This trial in 16,949 AMI patients showed that the 30-day mortality rates for single-bolus tenecteplase and accelerated alteplase were almost identical (6.18 % for tenecteplase and 6.15 % for alteplase) (Assessment of the Safety and Efficacy of a New Thrombolytic (ASSENT-2) Investigators 1999). Intracranial hemorrhage rates were similar in both groups (0.9 %), but fewer noncerebral hemorrhages and a lower need for blood transfusion were noted in the tenecteplase group. In conclusion, tenecteplase and 90-min alteplase are equivalent in terms of mortality and rates of intracranial hemorrhage. The single-bolus regimen for tenecteplase may facilitate thrombolytic therapy.

Safety Concerns

Tenecteplase is contraindicated in cases of active internal bleeding, history of cerebrovascular accident, recent intracranial or intraspinal surgery or trauma, intracranial neoplasm, arteriovenous malformation, or aneurism, in cases of known bleeding diathesis, or in severe uncontrolled hypertension.

The most common complication during tenecteplase therapy is bleeding. In clinical studies of tenecteplase, patients were treated with both aspirin and heparin. Heparin may contribute to the bleeding risk. Use of tenecteplase with other antiplatelet agents has not been adequately studied. In the ASSENT-2 study, the incidence of intracranial hemorrhage was 0.9 % and any stroke was 1.8 %

■ Lanoteplase

Lanoteplase (also referred to as ΔFE1X PA, BMS-200980, SUN9216, and nPA) is currently not commercialized. Lanoteplase is a t-PA variant in which the fibronectin fingerlike and epidermal growth factor domains have been removed (Collen et al. 1988). In addition, an asparagine to glutamine substitution at amino acid 117 provides reduced clearance (Hansen et al. 1988). The clearance appears to be mediated by the low-density lipoprotein receptor-related protein and asialoglycoprotein receptors, and the mannose receptor plays a smaller role (Komoriya et al. 2007). The clinical pharmacokinetics of lanoteplase is summarized in Table 15.7.

The Intravenous nPA for Treatment of Infarcting Myocardium Early (InTIME) study compared lanoteplase with accelerated alteplase. Patients were randomized to receive intravenous bolus doses of 15, 30, 60, or 120 kU/kg (not to exceed 12,000 kU) of lanoteplase or accelerated alteplase (den Heijer et al. 1998). A statistically significant increase in the proportion of patients with TIMI grade 3 flow at 60 min was noted with increasing lanoteplase dose ($p < 0.001$). There was no difference in the 30-day composite end point of death, heart failure, major bleeding, or nonfatal infarction (Ross 1999).

A larger randomized, multicenter equivalence trial (InTIME-II) in 15,078 patients compared 120 kU/kg lanoteplase with accelerated alteplase (The InTIME Investigators 2000). The primary end point was 30-day mortality with an incidence of 6.75 % for lanoteplase and 6.61 % for alteplase. The incidence of stroke was not statistically significantly different between treatment groups (1.87 % for lanoteplase and 1.53 % for alteplase). The incidence of hemorrhagic stroke was 0.64 % for alteplase and 1.12 % for lanoteplase ($p = 0.004$).

CONCLUSIONS

Recombinant technology has brought about significant advances in the treatment of coagulation disorders and in the availability of thrombolytic agents for the treatment of thrombotic disorders.

Current efforts focus on modifying the proteins to enhance their pharmacokinetic properties and to reduce immunogenicity. For recombinant coagulation factors, this includes pegylation or incorporating into pegylated liposomes, polysialylation, and attachment to the Fc region of IgG to increase half-life or mutagenesis to enhance resistance to degradation or clearance. Notably, cost continues to be a significant limitation in making recombinant coagulation factors available to all patients. Potentially, the development of biosimilars and a clear regulatory pathway to make these available may ease this limitation in the future.

Although significant strides have been made in reducing mortality due to acute myocardial infarction, there continue to be some drawbacks with current thrombolytic agents including the need for large doses, limited fibrin specificity, and risk of bleeding and reocclusion. Future efforts will focus on developing recombinant thrombolytic agents to improve efficacy and safety such as selective antibody-targeted plasminogen activators and chimeric molecules and to develop effective agents and regimens for the treatment of stroke and pulmonary embolism.

SELF-ASSESSMENT QUESTIONS

■ Questions

Question 1: A number of second-generation thrombolytic agents have either been approved or are in late stages of development. Discuss some of the limitations that the second-generation thrombolytic agents are designed to address.

Question 2: Design a rFVIII therapeutic regimen for a 35-kg patient with a laceration. Assume that the desired plasma concentration of factor VIII is 30 IU/dL.

Question 3: What criteria should factor VIII dosage be based on?

■ Answers

Answers 1: Although alteplase demonstrated an increased patency rate in the infarct-related artery and a decrease in mortality, several areas were identified where further improvements could be made in the treatment of acute myocardial infarction. Second-generation thrombolytic agents are designed to address some of these shortcomings.

(i) Due to the rapid clearance of rt-PA from the circulation by the liver, the current administration is via intravenous infusion over 90 min or 3 h. Second-generation thrombolytic agents have a slower plasma clearance allowing administration as a single- or double-bolus regimen (see Tables 15.1, 15.2, 15.3, 15.4, 15.5, 15.6, and 15.7).

(ii) Although alteplase is more fibrin selective compared to streptokinase and urokinase, there is still a 30–50 % fall in systemic fibrinogen levels. Second-generation thrombolytic agents are more fibrin specific and could result in further reduction in systemic fibrinogenolysis.

Answers 2: Dose = 30 IU/dL × 50 mL/kg (volume of distribution) × 35 kg = 525 IU.

Answers 3: Dosage should be individualized based on the needs of the patient, severity of deficiency, presence of inhibitors, and the desired increase in factor VIII.

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