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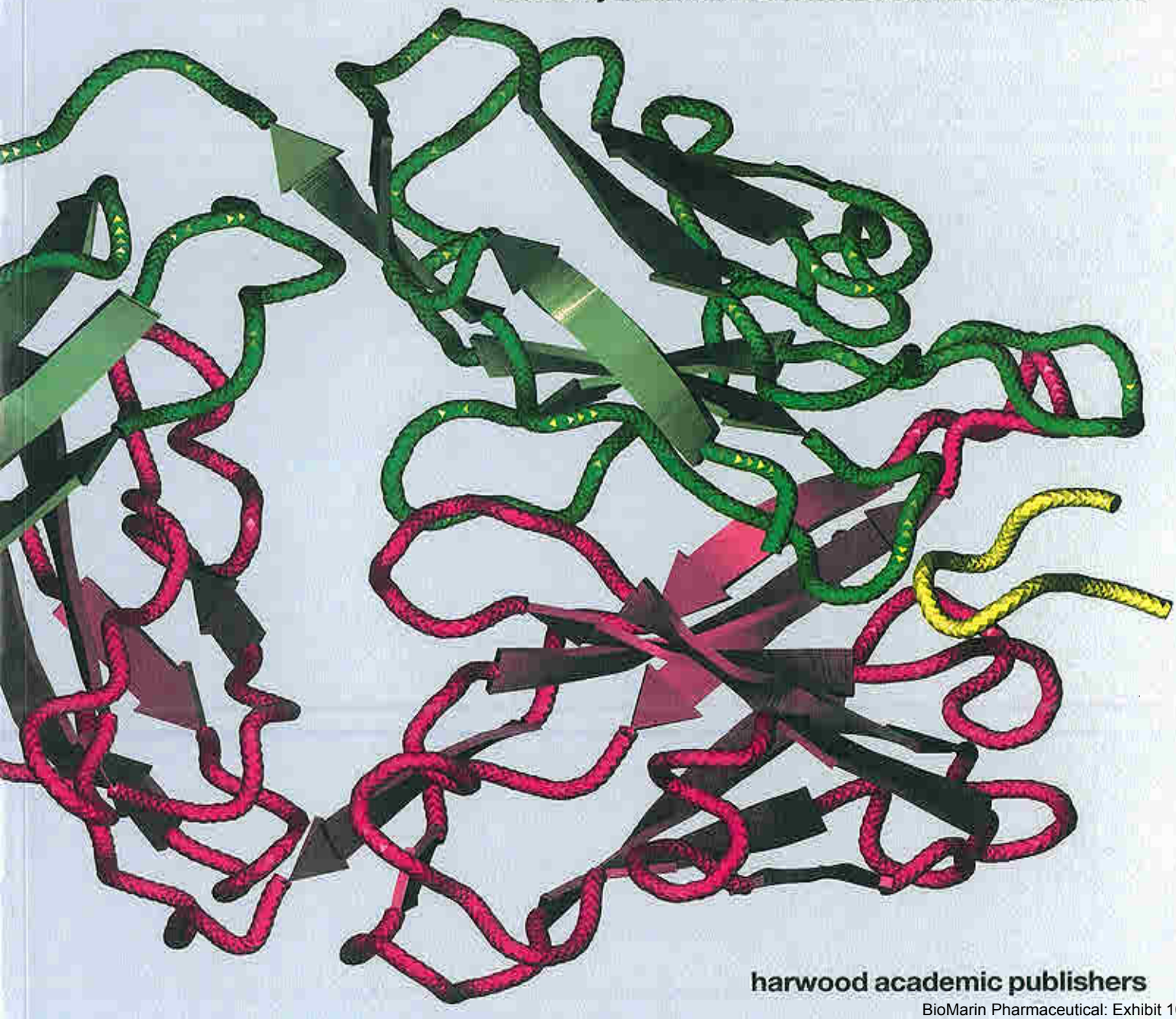
Pharmaceutical Biotechnology

An Introduction for Pharmacists and Pharmaceutical Scientists

Edited by Daan J A Crommelin and Robert D Sindelar



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The cover illustration shows a ribbon representation of the antigen binding fragment of bactericidal antibody MN12H2 in complex with a synthetic peptide. The peptide is derived from the top of extracellular loop 4 of outer membrane protein PorA of *Neisseria meningitidis* (strain H44/76). Heavy and light chain of the antibody are shown in magenta and green and the peptide is shown in yellow. This model is based on an X-ray crystallography study performed by Jean van den Elsen (Antibody Recognition of *Neisseria meningitidis*: a thermodynamic and structural analysis of the interaction between a bactericidal monoclonal antibody and a class 1 outer membrane epitope (1996), thesis, Utrecht University).

14 Recombinant Tissue-Type Plasminogen Activator and Factor VIII

by: Nishit B. Modi

Introduction

Coagulation and fibrinolysis normally exist in a mutually compensatory or balanced state. Endogenous regulatory mechanisms ensure that the process of hemostasis and blood coagulation at a site of injury and the subsequent fibrinolysis of the blood clot is normally localized and well controlled. This ensures a rapid and efficient hemostastic response at the 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 the therapeutic aspects of recombinant tissue-type plasminogen activator (rt-PA) and Factor VIII which are now available through recombinant technology.

Tissue-Type Plasminogen Activator

Introduction

Deposition of fibrin and platelets in the vasculature causes thromboembolic diseases which are responsible for considerable mortality and morbidity. Early thrombolytic therapy can decrease mortality and improve coronary artery patency in patients with an acute myocardial infarction (AMI) (Bates and Topol, 1989). During fibrinolysis, the inactive zymogen plasminogen is enzymatically converted to the active moiety, plasmin, by various physiologic plasminogen activators, such as tissue-type plasminogen activator and single-chain urokinase-type plasminogen activator (scu-PA). Plasmin subsequently digests the insoluble fibrin matrix of a thrombus to yield soluble fibrin degradation products (Figure 14.1). Tissue plasminogen activator (t-PA) exhibits fibrin-specific plasminogen activation (Hoylaerts *et al.*, 1982), 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. Furthermore, t-PA is not associated with the allergic and hypotensive effects reported for the non-endogenous plasminogen activators, streptokinase and acylated plasminogen-streptokinase activator complex (APSAC).

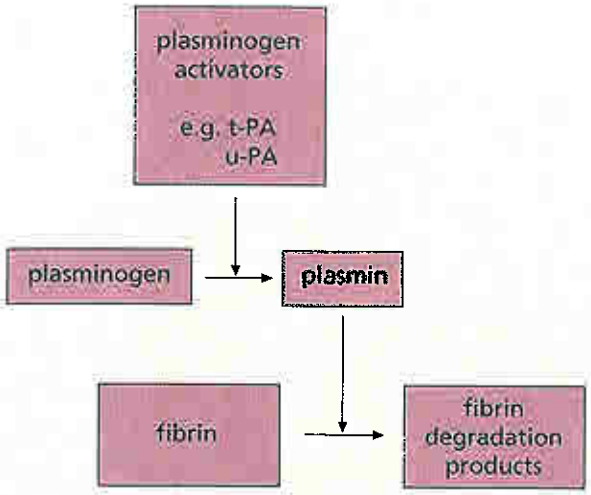

Figure 14.1. Schematic representation of the fibrinolytic pathway.

Structure

Native tissue-type plasminogen activator is a serine protease synthesized by vascular endothelial cells as a single chain polypeptide of 527 amino acids and has a molecular mass of 64 kD (Pennica *et al.*, 1983). Approximately 6–8% of the molecular mass consists of carbohydrate. A schematic primary structure of human t-PA is shown in Figure 14.2. There are seventeen disulfide bridges and four 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 moiety at Asp184 have been characterized (Bennett, 1983). Type I t-PA is glycosylated at asparagines 117, 184 and 448 whereas Type II t-PA lacks a glycosylation at asparagine 184. The asparagine at 218 is normally not occupied in either form of t-PA (Vehar *et al.*, 1984a). Asparagine 117 contains a high

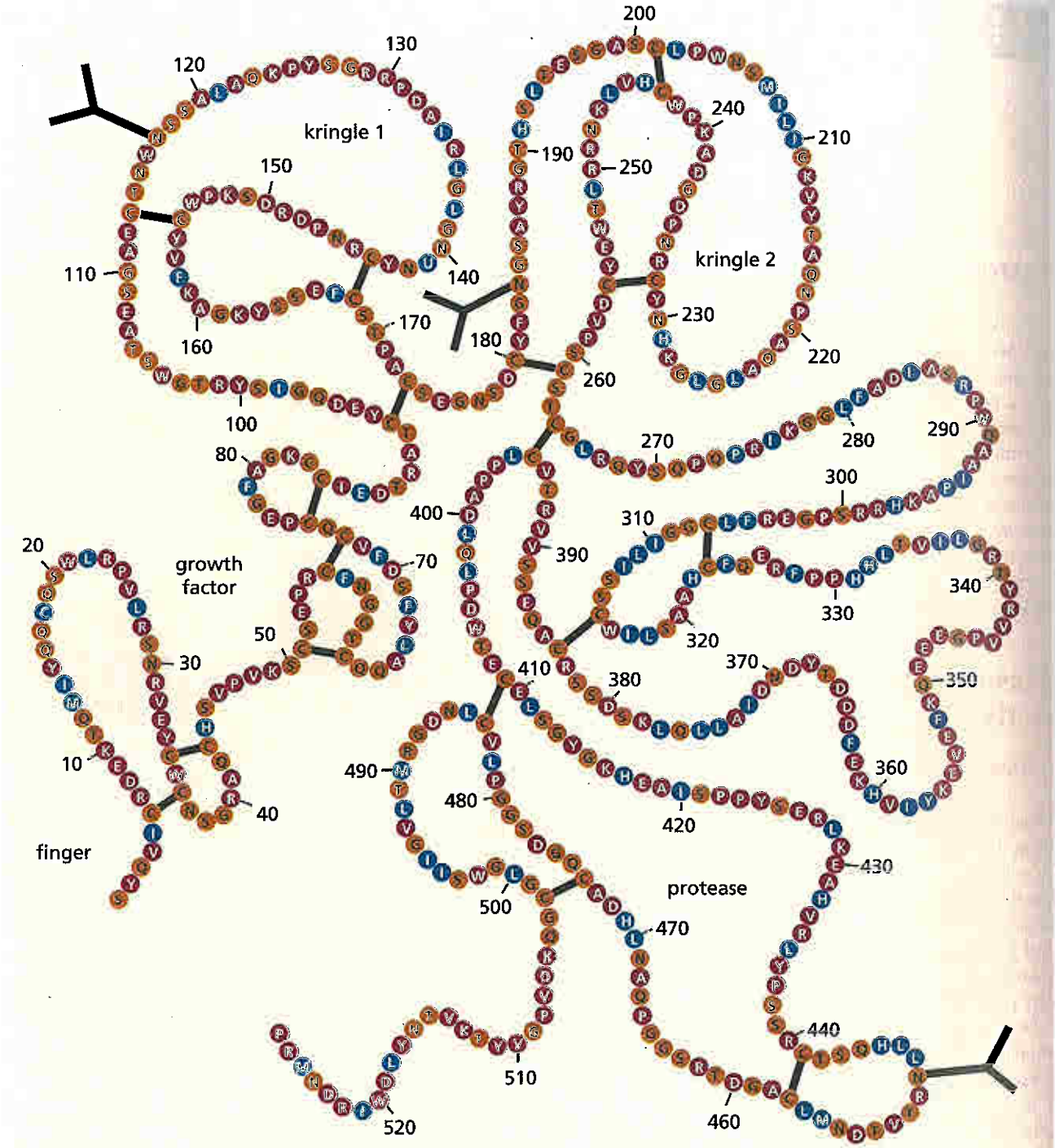

Figure 14.2. Primary structure of tissue-plasminogen activator.

mannose oligosaccharide whereas Asn184 and Asn448 are of the complex carbohydrate type (Spellman *et al.*, 1989). Complex N-linked glycan structures are ones containing a disaccharide Galβ(1,4)GlcNac and terminate in sialic acids while an oligomannose (high mannose) type glycan contains only mannose in the outer arms (see recommended reading for further details). Type II t-PA has a slightly higher specific activity *in vitro* compared with type I t-PA

(Einarsson *et al.*, 1985). During fibrinolysis, the single chain polypeptide is cleaved between Arg275 and Ile276 by 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 two 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).

Recombinant t-PA (rt-PA)

Recombinant t-PA (alteplase) is identical to endogenous human t-PA. Like melanoma-derived t-PA, rt-PA lacks a glycosylation at Asn218 and exists as two forms differing by the absence or presence of a carbohydrate at residue Asn184 (Vehar *et al.*, 1986). Initially, rt-PA was produced as a two-chain form in Chinese Hamster Ovary (CHO) cells using a roller bottle (RB) process. Most of the initial pharmacology and clinical studies were conducted using rt-PA derived from this process. Subsequently, a large scale suspension culture (SC) process that produced primarily (80%) single chain rt-PA was developed for commercialization. A pharmacokinetic comparison of rt-PA derived from the RB and SC processes showed that the SC-derived rt-PA was cleared from the circulation approximately 30% faster than RB-derived rt-PA (Data on file, Genentech, Inc.). However, when used at pharmacokinetically similar doses, both had similar pharmacodynamic and therapeutic properties.

Pharmacology

During fibrinolysis the inactive zymogen plasminogen is converted to the active enzyme plasmin by plasminogen activators. Plasmin then degrades the insoluble fibrin clot into soluble degradation products. Tissue plasminogen activator has a high affinity for fibrin and is a strong activator of plasminogen. Tissue plasminogen activator is a poor enzyme in the absence of fibrin, however, there is a > 600-fold enhancement in the activation of plasminogen by t-PA in the presence of fibrin (Hoylaerts *et al.*, 1982). Plasminogen activators are inhibited by the action of plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2), which normally circulate at a concentration of 5–20 μg/L in the plasma (Collen and Lijnen, 1994). Mean t-PA antigen concentrations at rest in humans are approximately 5 μg/mL (Holvoet *et al.*, 1985) and can increase about 1.5–2-fold in venous occlusion (Holvoet *et al.*, 1987). Circulating plasmin is rapidly inactivated by α_2-antiplasmin whereas plasmin formed on the fibrin clot surface is only slowly inactivated. This allows for efficient plasminogen activation at the target site (the fibrin clot) with less systemic activation and fewer systemic side effects compared to other thrombolytic agents currently approved for the management of acute myocardial infarction. If the amount of plasmin produced is sufficient to deplete the available α_2-antiplasmin, a systemic fibrinolytic state, characterized by activation of plasminogen, depletion of α_2-antiplasmin, decreased fibrinogen levels and increased fibrinogen degradation products, can occur. This can subsequently lead to hemorrhagic complications. Decreases in plasma levels of fibrinogen (to 54–61% of baseline), plasminogen (to 54–70% of baseline), and α_2-antiplasmin (to 25–35% of baseline) have been reported following 100 mg rt-PA over 1.5–3 hours (Seifried *et al.*, 1989; Tanswell *et al.*, 1992). These effects had reverted to 70–88% of baseline levels by 24 hours after treatment.

Disposition of rt-PA

The pharmacokinetics of rt-PA have been studied in mice, rats, rabbits, dogs, primates, and humans. After intravenous administration, the plasma concentrations decline rapidly with an initial dominant half-life of less than 5 minutes in all species. The clearance ranges from 27 mL/min in rabbits (Hotchkiss *et al.*, 1988), 29 mL/min in monkeys (Baughman, 1987), and 0.62 L/min in humans (Tanswell *et al.*, 1989). Recombinant t-PA exhibits non-linear pharmacokinetics at high plasma concentrations (Tanswell *et al.*, 1990). The estimated Km and Vmax computed by simultaneously fitting multiple plasma concentration-time curves following several different doses were 12–15 μg/mL and 3.7 μg/mL/hr, respectively, with little species variation in these parameters. The pharmacokinetics are essentially linear in cases where the plasma concentration does not exceed 10–20% of Km (i.e. 1.5–3 μg/mL). A pharmacokinetic summary of rt-PA following intravenous administration in humans is presented in Table 14.1. There was no difference in the pharmacokinetics following the different regimens. Recombinant t-PA has an initial volume of distribution that approximates plasma volume, and shows a rapid plasma clearance. The initial half-life was less than 5 minutes.

The primary route of rt-PA clearance is via receptor-mediated mechanisms in the liver. Three liver cell types 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 (Otter *et al.*, 1992). Hepatocytes clear t-PA via a carbohydrate-independent, receptor-mediated mechanism. Recent data suggests that this carbohydrate-independent clearance is mediated via the low density lipoprotein receptor-related protein (LRP) (Bu *et al.*, 1993).

Reference	Administration regimen	Health status	Cmax (μg/mL)	CL (L/min)	V_1 (L)	V_{ss} (L)	$t_{1/2\beta}$ (min)	$t_{1/2\beta}$ (min)
Tanswell et al, 1989	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
	0.5 mg/kg/30 min	Healthy	1.8±0.25	0.60±0.09	4.3±0.8	8.0±0.9	4.4±0.4	40±3.1
Seifried et al, 1989[b)]	100 mg/2.5 hr[c)]	AMI	3.3±0.95	0.38±0.07	2.8±0.9	9.3±5.0	3.6±0.9	16±5.4
Tanswell et al, 1992	100 mg/1.5 hr[d)]	AMI	4±1	0.57±0.1	3.4±1.5	8.4±5	3.4±1.4	72±68

Table 14.1. Clinical pharmacokinetic profile of rt-PA following intravenous administration in healthy volunteers and patients with acute myocardial infarction (AMI). All data reported are based on an immunoreactive assay.[a)]
[a)] Cmax = maximum rt-PA concentration; CL = plasma clearance; V_1 = initial volume of distribution; V_{ss} = Steady-state volume of distribution; t1/2 = half-life 3-compartment model was used. The β half-life corresponds to the second phase. [b)] A 3-compartment model was used. The β half-life corresponds to the second phase. The terminal half-life was 3.7 ± 1.4 hr. [c)] 10 mg bolus, 50 mg over 1 hr, then 30 mg in 1.5 hr. Note that the second infusion is of a shorter duration but at a similar rate to the standard dosage regimen. [d)] 15 mg bolus, 50 mg over 0.5 hr, then 35 mg in 1 hr.

Several reports have shown a correlation between a change in hepatic blood flow and plasma concentration of thrombolytic agents in healthy volunteers. The therapeutic implications of pharmacokinetic and pharmacodynamic drug interactions with thrombolytic agents were recently reviewed (De Boer *et al.*, 1995). Clinical data correlating rt-PA plasma concentrations in patients with acute myocardial infarction and hepatic blood flow are currently lacking and it is unclear if dose adjustments of thrombolytics are therapeutically necessary in patients receiving drugs that might affect liver blood flow.

Pharmaceutical Considerations

Recombinant human t-PA (Alteplase, Activase®, Genentech, Inc; Actilyse®, Boehringer Ingelheim) is supplied as a sterile, white to off-white lyophilized powder. Recombinant t-PA is practically insoluble in water and arginine is included in the formulation to increase the aqueous solubility. Phosphoric acid and/or sodium hydroxide may be used prior to lyophilization 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 should be protected from excessive light.

The powder is reconstituted by adding the accompanying Sterile Water for Injection, USP to the vial resulting in a colorless to pale yellow transparent solution containing 1 mg/mL rt-PA, with a pH of 7.3 and osmolality of approximately 215 mOs/kg. Recombinant t-PA is stable in solution over a pH range of 5 to 7.5. Since the reconstituted solution does not contain any preservatives, it should be used within eight hours of preparation and should be refrigerated prior to use. 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 may be diluted further in an equal volume of 0.9% Sodium Chloride for Injection, USP or 5% Dextrose Injection, USP to yield a concentration of 0.5 mg/mL and is compatible with glass bottles or poly-vinyl chloride bags.

Clinical Application

Recombinant human t-PA is indicated for use in the management of acute myocardial infarction and acute massive pulmonary embolism in adults. Two dosage regimens have been studied in patients experiencing acute myocardial infarction (AMI). Initially, rt-PA was administered as a 100 mg dose over 3 hours with a reduction in dosage for patients weighing less than 65 kg. In this regimen, 10 mg was administered as a bolus with 50 mg being infused over 1 hour, then 40 mg was infused over the subsequent 2 hours. Infarct artery-related patency rates of 70 to 77% are achieved at 90 minutes with this 3-hour regimen (Verstraete *et al.*, 1985; Carney *et al.*, 1992). Patency grades of blood flow in the artery are defined by the Thrombolysis in Myocardial Infarction (TIMI) trial and are assessed angiographically with TIMI grade 0 representing no flow; grade 1, minimal flow; grade 2, sluggish flow; and grade 3 indicating complete or full, brisk flow. Recently, the efficacy of an accelerated rt-PA regimen compared to streptokinase was demonstrated in 41,000 AMI patients (The GUSTO Investigators, 1993a,b). In the accelerated regimen, a bolus dose of 15 mg is administered over 2 minutes, followed by an infusion of 50 mg over 30 minutes. A dose of 35 mg is infused over the subsequent 60 minutes. For patients weighing less than 67 kg, a weight adjusted regimen is recommended with a bolus of 15 mg, followed by

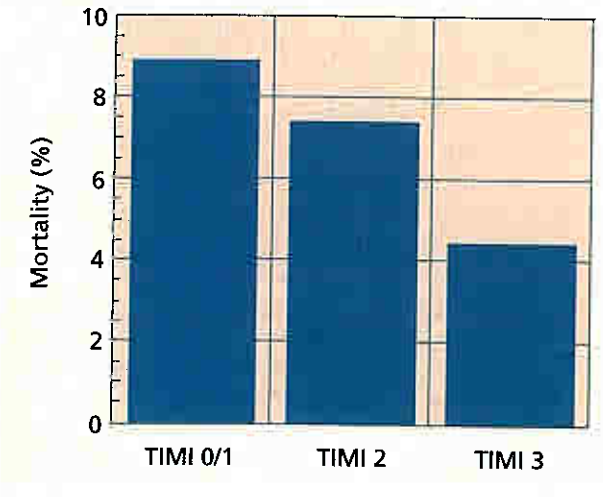

Figure 14.3. Correlation between TIMI flow and mortality demonstrating a decrease in mortality with improvement in TIMI flow.

0.75 mg/kg over 30 minutes, not to exceed 50 mg and then a rate of 0.5 mg/kg over 60 minutes, not to exceed 35 mg. The comparative efficacy of the two rt-PA regimens has not been evaluated. The GUSTO trial demonstrated a higher infarct-related artery patency rate (90-minute patency rate of 81%) and reduced mortality (an additional 10 lives saved per 10,000 patients treated) in the group treated with rt-PA with IV heparin compared to streptokinase with either subcutaneous or intravenous heparin (90-minute patency of 54–60%) (The GUSTO Investigators, 1993b). The rt-tPA group had an approximate 1% rate of intracranial hemorrhage. The GUSTO trial also indicated a decline in 30-day mortality with improvement in TIMI flow regardless of treatment assignment (Figure 14.3).

Contraindications

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

Second Generation Thrombolytic Agents

The rapid clearance of rt-PA from the circulation by the liver necessitates that it be administered as an intravenous infusion. Considerable preclinical and clinical research is currently underway to develop a rt-PA variant that is fibrin-specific and can be administered as an intravenous bolus injection. Several reviews (e.g. Higgins and Bennet, 1990) have outlined the progress of these efforts. Strategies that have been used to develop these rt-PA variants include domain deletions, glycosylation changes, or site directed amino acid substitutions (see Chapter 5). Several of these rt-PA variant have shown efficacy in animal models following bolus administration and are currently undergoing extensive clinical trials, hence, their availability is several years away.

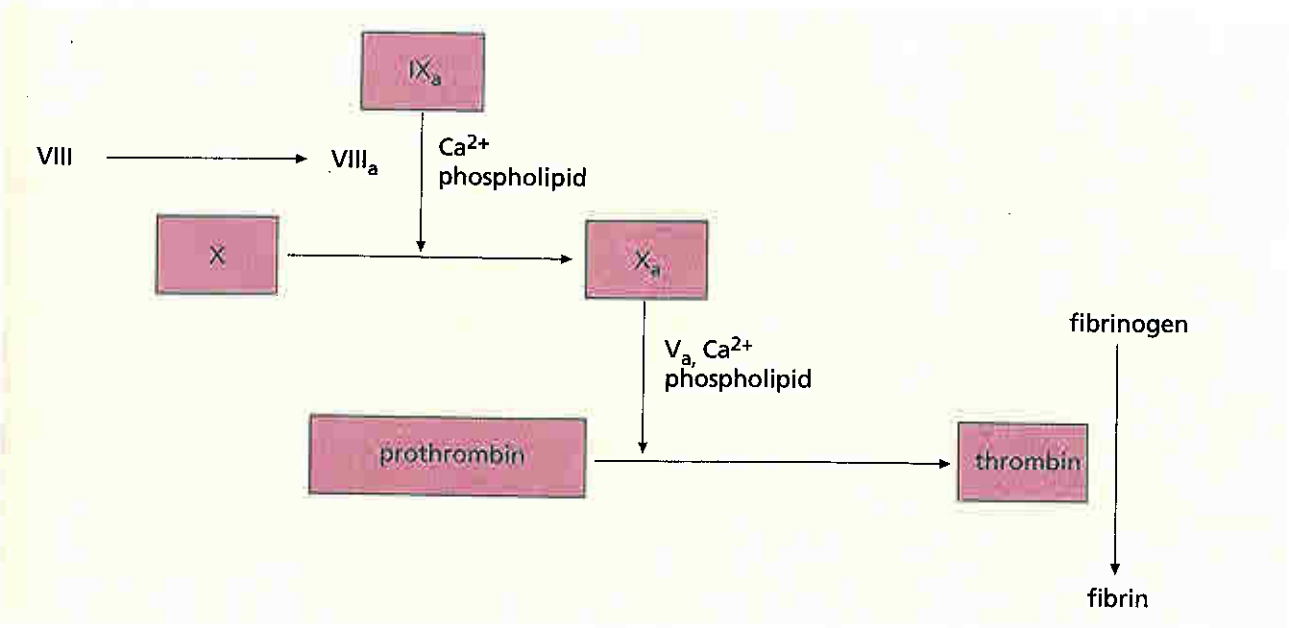

Figure 14.4. Simplified scheme showing the role of Factor VIII in the coagulation pathway.

Factor VIII

Factor VIII (antihemophilia factor) is a plasma protein that functions as a cofactor by increasing the Vmax in the activation of Factor X by Factor IXa in the presence of calcium ions and negatively charged phospholipid (Figure 14.4) (Jackson and Nemersen, 1980). The congenital absence of Factor VIII is characterized by a bleeding disorder termed Hemophilia A, an X-linked recessive disorder that afflicts approximately 1 in 10,000 males (Antonarakis *et al.*, 1987). The introduction of Factor VIII concentrates derived from plasma increased the quality of life and the life expectancy of individuals with hemophilia A, however, reliance on plasma as a source for Factor VIII also exposed patients to alloantigens and transfusion-associated viral disease (Schwartz *et al.*, 1990). Factor VIII derived from recombinant technology has the potential to eliminate many of the shortcomings of plasma derived antihemophilia factor and be available in an unlimited supply.

Factor VIII Structure

Factor VIII is synthesized as a single chain polypeptide of 2332 amino acids (Eaton *et al.*, 1987). Shortly after synthesis, cleavage occurs and most plasma Factor VIII circulates as a 80 kD light chain associated with a series of about 210 kD heavy chains in a metal ion-dependent complex. There are 25 potential N-linked glycosylation sites and 22 cysteines (Vehar *et al.*, 1984). The about 210 kD heavy chain is further proteolytically cleaved to a series of proteins of Mr 90–188 kD (Eaton *et al.*, 1986). The 90–210 kD proteins form a complex with the light chain that is mediated by a metal ion (Eaton *et al.*, 1986).

Recombinant Factor VIII

Recombinant Factor VIII from Baxter Healthcare (Recombinate®) is produced in a batch refeed culture process using transfected CHO cells whereas that from Bayer/Miles Inc (Kogenate®) is produced in a continuous cell culture process using transfected Baby Hamster Kidney cells. A major difference between recombinant Factor VIII from Bayer/Miles and Baxter is the presence of a Galα1->3Gal carbohydrate moiety in the Baxter product (Hironaka *et al.*, 1992). Recombinant factor VIII consists of multiple peptides including an 80 kD protein and various extensions of about 90 kD subunit protein (Schwartz *et al.*, 1990).

Pharmacology

The concentration of Factor VIII in plasma is about 200 ng/mL (Hoyer, 1981). It is not known with certainty where Factor VIII is synthesized. There is evidence 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 allowing localization of von Willebrand factor and Factor VIII.

Factor VIII circulates in blood as a large precursor polypeptide devoid of coagulant activity. Cleavage by thrombin at Arg372-Ser373, Arg740-Ser741, and Arg1689-Ser1690 results in procoagulant function (Vehar *et al.*, 1984b). 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, shortly after synthesis, the single chain polypeptide is cleaved and most of the factor VIII in plasma exists as a 80 kD light chain and a series of heavy chains. The heavy and light chains circulate as a population of heterodimers that require a divalent cation to maintain their interaction and procoagulant potential.

Disposition of Recombinant Factor VIII

Several studies have evaluated the clinical pharmacokinetics of recombinant factor VIII. The pharmacokinetic profile is summarized in Table 14.2. Following administration, the increase in Factor VIII concentration is dose proportional and the disposition is similar following a single and chronic dosing. The terminal half-life is 14–16 hours and the initial volume of distribution approximates plasma volume.

Pharmaceutical Considerations

Recombinant Factor VIII (Kogenate®, Bayer/Miles; Recombinate®, Baxter) is supplied as sterile, single dose vials containing 250 to 1000 IU of Factor VIII activity. The preparation is stabilized with human albumin and is lyophilized. The product contains 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 as the diluent bottle may break. Factor VIII is reconstituted with the diluent provided. The reconstituted product must be administered intravenously by either direct syringe injection or drip infusion within 3 hours of reconstitution.

Clinical Application

Recombinant Factor VIII, antihemophilic factor, (Recombinate®, Kogenate®) is indicated for the treatment of classical hemophilia (hemophilia A), for the prevention and

Reference	Administration regimen	CL (mL/hr/kg)	V (mL/kg)	$t_{1/2}$ (hr)	MRT (hr)
Morfini et al, 1992	50 U/kg Recombinate	3.1±1.2	62±18[a]	14.5±4.3	NR[b]
Schwartz et al, 1990[c]	50 IU/kg Kogenate	2.5±0.8 2.6±0.9 2.2±0.7	51±13[d] 48±12 49±18	15.8±3.9 13.9±3.2 16.6±3.0	21.2±5.3 19.1±4.5 22.6±4.1
Harrison et al, 1991	50 U/kg Kogenate	2.4±0.7	51±13	16.5±3.5	22.2±4.9

Table 14.2. Clinical pharmacokinetic profile of recombinant factor VIII following intravenous administration in patients with hemophilia A. [a] $V_{d\text{-}area}$; [b] Not reported; [c] Data reported for Weeks 1, 13 and 25; [d] V_{ss}.

control of hemorrhagic episodes and in the perioperative management of patients with hemophilia A. Since recombinant antihemophilic factor is indicated in the treatment of bleeding disorders arising from a deficiency in factor VIII, it is essential that this deficiency be identified before administration of rAHF. Recombinant Factor VIII is also approved for the treatment of hemophilia A in certain patients with inhibitors to Factor VIII. Recombinant factor VIII is not indicated for the treatment of von Willebrand disease. Factor VIII replacement should be based on the minimal effective hemostatic concentration necessary for treatment, the volume of distribution and the elimination half-life.

Schwartz *et al.* (1990) investigated the pharmacokinetics, safety and efficacy of recombinant factor VIII in patients with hemophilia A. In this study, the mean residence time and elimination half-life of recombinant factor VIII were equal to or exceeded that for plasma derived factor VIII, whereas the clearance and steady-state volume of distribution for recombinant factor VIII were slightly smaller than that for plasma derived factor VIII. In the safety and efficacy portion of the study, the mean incremental recovery of factor VIII following 50 IU/kg of recombinant factor VIII given at weeks 1, 5, 9, 13, and 25 was 2.49–2.92% per IU administered per kilogram and was not statistically dependent on multiple dosing.

The incidence of formation of inhibitors to recombinant factor VIII in previously untreated patients is approximately 20% (Lusher *et al.*, 1993). This incidence rate is similar to that for plasma derived factor VIII, where approximately 10–15% of patients treated develop antibodies which neutralize factor VIII and result in resistance to treatment (McMillan *et al.*, 1988). A direct comparison of incidence rates is difficult since studies investigating the incidence of inhibitor development to recombinant factor VIII used previously untreated patients whereas those investigating plasma derived factor VIII were conducted in previously treated patients, a group that may be at lower risk for inhibitor development (Lusher *et al.*, 1993). The study by Lusher *et al.* (1993) showed that the risk of developing antibodies to recombinant factor VIII correlated with the severity of the disease and the intensity of exposure to factor VIII. Inhibitors were detected 1 to 15 months after the initial exposure with factor VIII. In several of the patients in whom inhibitors were detected, they disappeared completely or remained at a low level (≤ 10 Bethesda units) despite continued treatment with factor VIII.

Contraindications

Since trace amounts of mouse or hamster protein may be present in recombinant Factor VIII as contaminants from the expressions system, caution should be exercised when administered to individuals with known hypersensitivity to plasma-derived antihemophilic factor or with hypersensitivity to biological preparations with trace amounts of murine or hamster proteins.

Concluding Remarks

This chapter has provided an overview of two hematologic products currently available throughout recombinant technology that have made a significant impact on patient care. Several other recombinant products that could have a further clinical benefit (e.g., second generation thrombolytics, Factor IX) are currently under investigation. ■

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Self-Assessment Questions

Question 1: *Several biopharmaceutical companies are developing second generation thrombolytic agents. What are the motivations for this ?*

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

Answers

Answer 1: While rt-PA has demonstrated an increase in the patency rate of the infarct-related artery and a decrease in the mortality, there are potentially several areas where further benefits can be made in the treatment of acute myocardial infarction :

(i) Due to the rapid hepatic clearance of rt-PA from the circulation, currently administration is via an intravenous infusion regimen to maintain therapeutic concentrations. Several of the second generation agents are claimed to have a longer half-life and slower plasma clearance allowing administration via a single or double bolus regimen (cf. Figure 5.18).

(ii) Although rt-PA results in fewer systemic side effects compared to other thrombolytic agents, there is an approximate 30–50% fall in systemic fibrinogen levels. A more fibrin specific second generation thrombolytic could result in a further reduction in systemic fibrinogenolysis and potentially a reduction in the incidence of intracranial hemorrhage.

Answer 2: Dose = 30 U/dL × 50 mL/kg (volume of distribution) × 30 kg = 450 IU.