Chapter 10
Physiological Functions of Plasminogen Activation: Effects of Gene Deficiencies in Humans and Mice

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Abstract Exhaustive analysis of humans and mice with genetic deficiencies in plasminogen, plasminogen activators, plasmin inhibitor, and plasminogen activator inhibitors have yielded fundamental new insights into both the mechanisms of activation and the physiological functions of the plasminogen activation system. At least five different pathways for the activation of plasminogen are operative in vivo, and these five pathways display a remarkable functional redundancy. Plasminogen as well as the components that govern the activation and inhibition of the plasminogen activation system are dispensable for development. However, the cleavage of fibrin and other extracellular substrates by plasmin is critical for postnatal remodeling and repair of multiple epithelial and mesenchymal tissues. As a consequence, life without plasminogen is associated with high morbidity and mortality due to progressive multiorgan damage. Conversely, genetic deficiencies in inhibitors of plasmin and plasminogen activators not only markedly accelerate tissue repair but also result in a lifelong bleeding predisposition due to premature fibrin dissolution.

Introduction

The plasminogen activation system was the first proteolytic system to be implicated in human tumor progression, and for long it has served as a paradigm for extracellular proteolysis in cancer, as well as a prospective target for cancer therapy (Dano et al. 1985, 1999). Plasminogen, its activators, inhibitors, and cellular receptors

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have been exhaustively studied in the context of development, invasion, metastasis, and prognosis of human tumors, as reflected by the publication of more than 4,000 primary research publications and 600 review articles containing the keywords "plasminogen" and "cancer."

This chapter will describe some of the key insights into the physiological functions of plasminogen activation that have been gained over the last two decades from the study of humans and mice deficient in plasminogen, plasmin inhibitor, and plasminogen activator inhibitors. In the chapter, the term "physiological" is broadly defined as processes that can be regarded as evolutionarily beneficial (e.g., development, reproduction, restoration of tissue homeostasis). The many putative proteolytic and nonproteolytic functions of components of the plasminogen activation system that are unrelated to the activation of plasminogen will not be covered [for recent reviews of these exciting topics, *see*, e.g., Blasi and Carmeliet (2002), Loskutoff et al. (1999), Melchor and Strickland (2005), Stefansson and Lawrence (2003), Tsirka (2002), and Yepes and Lawrence (2004)].

The Components of the Plasminogen Activation System – A Short Synopsis

Plasminogen is a modular trypsin-like serine protease zymogen that is converted to the active protease plasmin, by a single endoproteolytic cleavage within the activation site of the serine protease domain. Plasminogen is predominantly synthesized by the liver and is present in a very high concentration (1–2 μm) in plasma and other extravascular fluids (Collen and Lijnen 1986). Lower level extrahepatic synthesis of plasminogen has also been documented (Zhang et al. 2002). Plasminogen is converted to the active protease plasmin by urokinase plasminogen activator (uPA), tissue-type plasminogen activator (tPA), and by an as yet unidentified serine protease (Lund et al. 2006). tPA and uPA are closely related trypsin-like serine proteases that are expressed at many extrahepatic sites, either constitutively or after disruption of homeostasis, leading to local activation of plasminogen (Dano et al. 1985). Following its formation, plasmin is inhibited primarily by α_2-antiplasmin: a fast-acting, serpin-type protease inhibitor that is synthesized by the liver and is present in high concentration in plasma and extravascular fluids (Coughlin 2005, Lijnen and Collen 1985). Three serpin-type inhibitors for uPA and tPA have been described: plasminogen activator inhibitor (PAI-1), PAI-2, and neuroserpin. Of these, PAI-1 appears to be the most critical physiological inhibitor of uPA and tPA, while the functions of PAI-2 and neuroserpin in the inhibition of plasminogen activation at present are unclear (Dougherty et al. 1999, Galliciotti and Sonderegger 2006, Loskutoff 1993, Yepes and Lawrence 2004). After inhibition by their cognate serpins, plasmin and plasminogen activators are internalized for lysosomal degradation by members of the low-density lipoprotein receptor family (Herz and Strickland 2001, Strickland et al. 1995).

The Many Pathways to Plasmin

Five pathways are currently known to lead to the conversion of plasminogen to plasmin during physiological conditions (Fig. 10.1). tPA is a poor activator of plasminogen in solution, but a very potent activator of plasminogen in the context of a fibrin clot. Fibrin strongly promotes tPA-mediated plasminogen activation by serving as a scaffold for the binding of tPA and plasminogen that brings the two

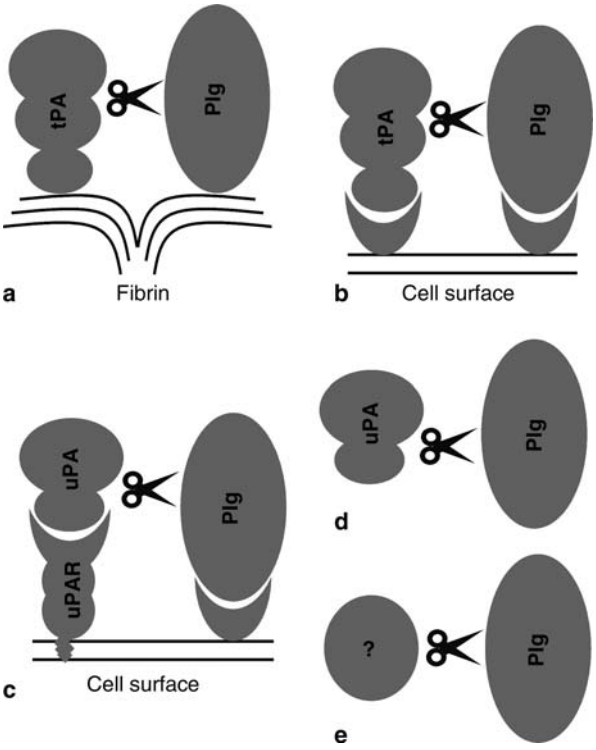

Fig. 10.1 The five known physiological pathways for plasminogen activation. **a** Fibrin-dependent activation of plasminogen (*Plg*) by tissue-type plasminogen activator (*tPA*). Fibrin serves as a scaffold for binding of tPA and Plg and protects newly formed plasmin from inactivation by α_2-antiplasmin. **b** Cell surface-mediated Plg activation by tPA. tPA and Plg bind to specific cell surface receptors that provide a spatially favorable alignment and productive plasmin generation. The cell surface protects plasmin from inactivation by α_2-antiplasmin. **c** urokinase plasminogen activator receptor (*uPAR*)-dependent Plg activation by urokinase plasminogen activator (*uPA*). Pro-uPA binds to uPAR and Plg binds to specific cell surface receptors leading to feedback activation of Plg to plasmin by uPA, and, conversely, pro-uPA to active uPA by plasmin. Cell surface-bound plasmin is protected from inactivation by α_2-antiplasmin. **d** uPAR-independent activation of Plg by uPA. uPA converts Plg to plasmin in a process that does not involve uPAR and may be cell surface dependent or independent. **e** uPA and tPA-independent Plg activation. Plg is proteolytically converted to plasmin by an unknown serine protease that is different from uPA and tPA

molecules in close apposition and by protecting plasmin from inactivation by α_2-antiplasmin (Collen 1980, Collen and Lijnen 2005, Hoylaerts et al. 1982, Thorsen 1992).

A second pathway for plasminogen activation by tPA is cell mediated and involves the simultaneous binding of tPA and plasminogen to the cell surface, which leads to productive plasmin generation. The annexin II-S100A10 heterotetramer recently has emerged as one strong candidate receptor for mediating physiologically relevant tPA-dependent cell surface plasminogen activation, as annexin II-deficient mice develop widespread spontaneous fibrin deposition and cells from annexin II-deficient mice display reduced tPA-mediated plasminogen activation in vitro (Beebe et al. 1989; Felez et al. 1991; Hajjar et al. 1987, 1986; Hajjar et al. 1994; Kim and Hajjar 2002; Ling et al. 2004; Plow et al. 1986).

A principal pathway for plasminogen activation by uPA involves the binding of uPA to a specific cell surface receptor, the urokinase plasminogen activator receptor (uPAR). uPA is synthesized as a single chain proenzyme (pro-uPA) with low intrinsic activity that is efficiently converted to active two-chain uPA by plasmin. Two-chain uPA, in turn, is a potent activator of plasminogen. The concomitant binding of pro-uPA to uPAR, and of plasminogen to as yet not fully characterized cell surface receptors strongly potentiates uPA-mediated plasminogen activation, probably through the formation of ternary complexes that align the two proenzymes in a way that exploits their low intrinsic activity and thereby favors a mutual activation process. The net result of this process is the efficient and localized generation of active uPA and plasmin on the cell surface (Ellis et al. 1991, Ellis and Dano 1993, Ellis et al. 1989, Ronne et al. 1991, Stephens et al. 1989).

Although uPAR appears to be critical for cell-mediated plasminogen activation by uPA in vivo (Liu et al. 2003, Liu et al. 2001), it is not the only physiologically relevant pathway, or even the dominant pathway, for the activation of plasminogen by uPA in mice. Thus, mice deficient in uPAR or mice with combined deficiencies in uPAR and tPA develop a much milder spectrum of phenotypic abnormalities than uPA-deficient mice or mice with combined uPA and tPA deficiencies. Furthermore, neither uPAR-deficient nor uPAR and tPA double-deficient mice display the pronounced defects in tissue repair that are characteristic of uPA-deficient, uPA and tPA double-deficient, or plasminogen-deficient mice (Dewerchin et al. 1996; Bezerra et al. 2001; Bugge et al. 1995a,b; 1996a,b Carmeliet et al. 1998, 1994; Deindl et al. 2003; Kitching et al. 1997; Ploplis et al. 1995; Shanmukhappa et al. 2006; *see* the following section). The dominant uPAR-independent pathway of plasminogen activation by uPA that was defined from these gene inactivation studies may involve as yet uncharacterized cellular receptors for uPA (Longstaff et al. 1999), or, alternatively, be cell-independent.

Recently, it was noted that skin wound healing was more severely impaired in plasminogen-deficient mice than congenic mice with combined uPA and tPA deficiencies. Furthermore, active plasmin and plasmin-α_2-antiplasmin complexes could be readily detected in extracts from uPA and tPA double-deficient wounds. This exciting finding demonstrates the existence of a third physiologically relevant pathway for plasminogen activation in mice (Lund et al. 2006). The protease

responsible for the activation of plasminogen in the combined absence of uPA and tPA is currently unknown, but appears to be an ecotin-inhibitable serine protease (Lund et al. 2006).

The various physiological pathways for plasminogen activation display significant functional redundancy in vivo. Thus, mice with single deficiencies in either uPA or tPA do not display the pervasive multiorgan pathology that befalls mice with combined uPA and tPA deficiency (Bugge et al. 1996a, Carmeliet et al. 1994, Drew et al. 1998). Furthermore, the capacity to repair injured tissues is generally much more severely affected in combined uPA and tPA-deficient mice than in mice with single deficiencies in the two plasminogen activators, which often display only modest impairments of tissue repair (Bezerra et al. 2001, Bugge et al. 1996a, Carmeliet et al. 1994, Kitching et al. 1997, Leonardsson et al. 1995, Shanmukhappa et al. 2006). In the central nervous system, however, uPA is expressed at very low levels, and tPA appears to be the dominant physiological plasminogen activator with little or no contribution by uPA under nonpathological conditions (Hoover-Plow et al. 2001, Mataga et al. 2004, Melchor et al. 2003, Mizutani et al. 1996, Nakagami et al. 2000, Oray et al. 2004, Pang et al. 2004, Wu et al. 2000).

Physiological Functions of Plasminogen Activation

Plasminogen deficiency is now established as the principal cause of ligneous conjunctivitis, a rare, multisyndromic, inherited disease that was described as early as 1847 (Bouisson 1847; Schuster et al. 1997, 1999b; Tefs et al. 2006). Critical insights into the physiological roles of plasminogen activation have been gained from the clinical examination of individuals with this disease. As of 2007, about 75 cases of severe or complete plasminogen deficiency in humans have been reported. These deficiencies are caused by homozygosity or compound heterozygosity for a wide assortment of missense, frameshift, splice site, and nonsense mutations that can be found throughout the plasminogen gene. The disease is characterized by the formation of chronic, disfiguring, wood-like (ligneous), fibrin-rich lesions on mucous membranes of multiple body sites. The onset and severity of lesion formation varies considerably from individual to individual. Ligneous lesions generally reappear rapidly after surgical removal due to defective wound healing. Tissues that can be affected include the conjunctiva and cornea of the eye, the gingiva, the ears, the sinuses, the larynx, the vocal cords, the bronchi, the gastrointestinal tract, the female genital tract, and the skin. Congenital occlusive hydrocephalus caused by floating thombi within the cerebrospinal fluid has also been described (Ciftci et al. 2003, Kraft et al. 2000, Ozcelik et al. 2001, Pantanowitz et al. 2004, Schott et al. 1998, Schuster et al. 1999a, Tefs et al. 2006; Table 10.1). Ligneous lesions are often quite debilitating, leading to impaired vision, tooth loss, chronic respiratory infections, infertility, and premature death (Baykul and Bozkurt 2004, Beck et al. 1999, Ciftci et al. 2003, Kraft et al. 2000, Ozcelik et al. 2001, Pantanowitz et al. 2004, Schuster et al. 1999a, Tefs et al. 2006). Interestingly, however, of about 75 patients with documented severe plasminogen deficiency

described in the literature, no episodes of venous thrombosis were documented. Histological examination of ligneous lesions often reveals extensive epithelial ulcerations with reactive hyperplasia, surrounding large amorphous, fibrin-rich masses that contain acute and/or chronic inflammatory cell infiltrates composed of neutrophils, T cells, macrophages, B cells, and mast cells. Neovascularization and deposition of plasma proteins such as immunoglobulin and albumin are frequently observed in ligneous lesions, whereas lipid, amyloid, and keratin are generally not detectable (Chambers et al. 1969, Cooper et al. 1979, Eagle et al. 1986, Gunhan et al. 1994, Hidayat and Riddle 1987, Holland et al. 1989, Mingers et al. 1997). Consistent with plasminogen deficiency as the underlying cause, ligneous lesions have been treated very efficiently by systemic or topical administration of plasmin, plasminogen, or even fresh-frozen plasma. This often leads to complete resolution of ligneous lesions (Schott et al. 1998, Tabbara 2004, Watts et al. 2002).

The precise etiology of the ligneous lesions that accompanies plasminogen deficiency in humans has not been definitively established. However, the pronounced accumulation of fibrin and inflammatory cells in ligneous lesions, when combined with data that plasminogen-deficient mice that are also genetically deficient in fibrinogen are completely protected from ligneous lesions, implicates insufficient extravascular fibrinolysis as the principal underlying cause. In this scenario, topical irritation, minor trauma, or infection of plasminogen-deficient mucus membranes triggers an inflammatory response that leads to local fibrin deposition. In the absence of sufficient extravascular fibrin clearance, a "vicious cycle" of fibrin-triggered inflammatory cell recruitment, and inflammatory cell-induced fibrin deposition occurs, causing the characteristic features of ligneous lesions. In summary, the study of the clinical effects of severe plasminogen deficiency in humans suggests a principal role of plasminogen activation in extravascular fibrin surveillance in multiple tissues, in particular those subjected to frequent trauma from environmental exposure.

Plasminogen-deficient mice display a spectrum of phenotypic abnormalities that are very similar to those observed in humans with plasminogen deficiency (Table 10.1). Fetal plasminogen is dispensable for mouse embryonic development, and plasminogen-deficient mice are generally unremarkable at birth (Bugge et al. 1995a, 1996b; Ploplis et al. 1995), although occlusive hydrocephalus has been noted in rare cases (Drew et al. 1998). However, like plasminogen-deficient humans, plasminogen-deficient mice with time develop focal lesions of multiple epithelial tissues, leading to wasting, impaired organ function, and premature death (Bugge et al. 1995a, 1996b; Drew et al. 1998; Ploplis et al. 1995). These lesions can be found in most organ systems and organs in the body, including the gastrointestinal tract (esophagus, squamous and glandular stomach, liver, pancreas, duodenum, colon, rectum), respiratory system (trachea, bronchi, lungs), female genital tract (vagina, uterus of parous females), eye (cornea, conjunctiva), and auditory system (middle ear, tympanic membrane, and external ear; Bugge et al. 1995a, 1996b; Drew et al. 1998; Eriksson et al. 2006; Ploplis et al. 1995). Plasminogen-deficient female mice display substantially diminished ability to nurture their litters due to impaired

mammary gland involution and impaired milk secretion, secondary to fibrin accumulation in the alveoli and ducts of the mammary gland (Green et al. 2006, Lund et al. 2000). Although the brain of plasminogen-deficient mice is anatomically normal, the mice display learning deficits that are associated with impaired long-term potentiation and synaptic plasticity (Hoover-Plow et al. 2001, Mataga et al. 2004, Mizutani et al. 1996, Nakagami et al. 2000, Oray et al. 2004, Pang et al. 2004).

The epithelial lesions that develop in plasminogen-deficient mice are histologically very similar to the epithelial lesions observed in plasminogen-deficient humans. They typically exhibit epithelial disruption with focal necrosis of underlying tissue, reactive hyperplasia, and extensive fibrin deposition with profuse inflammatory cell infiltration (Bugge et al. 1995a, 1996b; Drew et al. 1998; Eriksson et al. 2006; Ploplis et al. 1995). Impaired and aberrant tissue repair after chance trauma is likely to be the primary underlying cause of the spontaneous lesions that accumulate in plasminogen-deficient mice. Thus, studies of the kinetics and the overall outcome of the healing of defined injuries of plasminogen-deficient mice that are generated in a wide variety of epithelial and mesenchymal tissues have revealed a generalized and severe impairment of tissue repair (Table 10.1). The delay and aberrant healing documented in plasminogen-deficient mice include intravascular thrombus dissolution (Lijnen et al. 1996), incisional skin wounds (Lund et al. 1999, Romer et al. 1996), tympanic membrane perforation (Li et al. 2006), scrape and excimer-induced corneal wounds (Drew et al. 2000), experimental glomerulonephritis (Kitching et al. 1997), organic solvent-induced liver necrosis (Bezerra et al. 1999, Pohl et al. 2001), antigen-induced arthritis (Busso et al. 1998), skeletal muscle crush injury (Suelves et al. 2002), peripheral nerve injury (Akassoglou et al. 2000, 2002; Siconolfi and Seeds, 2001), neuronal remodeling after kainate-induced seizure (Wu et al. 2000), amyloid deposition in the brain (Melchor et al. 2003), and experimental myocardial infarction (Creemers et al. 2000).

Outside of the central nervous system, the impaired dissolution of fibrin appears to be the principal molecular defect that underlies the spontaneous multiorgan pathology and defective tissue repair associated with plasminogen deficiency in mice (Table 10.1). Thus, the genetic elimination of fibrinogen in plasminogen-deficient mice prevents wasting, normalizes the life span, and also completely prevents the formation of spontaneous lesions in the digestive tract, the respiratory tract, the urogenital tract, the cornea, the conjunctiva, and other tissues and organs (Bugge et al. 1996b, Drew et al. 1998). Likewise, with the notable exception of resolution of carbon tetrachloride-induced liver necrosis (Bezerra et al. 1999, Ng et al. 2001), fibrinogen gene disruption, or fibrinogen depletion have normalized the kinetics and overall outcome of the healing of all experimental tissue injuries where this has been tested. These include skin wound healing (Bugge et al. 1996b), corneal wound healing (Kao et al. 1998), antigen-induced arthritis (Busso et al. 1998), muscle regeneration (Suelves et al. 2002), and peripheral nerve damage (Akassoglou et al. 2000, 2002). Furthermore, heterozygosity for the fibrinogen gene markedly improved the lactational competence of plasminogen-deficient females (Green et al. 2006).

Table 10.1 Physiological effects of congenital plasminogen deficiency in humans and mice

Humans	
Ligneous lesions[a]	
Eyes (cornea, conjunctiva)	
Auditory canal (middle ear)	
Mouth (gingiva)	
Respiratory tract (sinuses, larynx, vocal cords, trachea, bronchi)	
Gastrointestinal tract (stomach)	
Female genital tract (vagina, cervix)	
Occlusive hydrocephalus	
Impaired fertility	
Impaired wound healing	
Increased mortality	
Mice	
Ligneous lesions	Fibrinogen dependence[b]
Eyes (cornea, conjunctiva)	Yes
Auditory canal (middle ear, tympanic membrane)	ND
Respiratory tract (trachea, bronchi, lungs)	Yes
Gastrointestinal tract (esophagus, stomach, liver, pancreas, duodenum, colon, rectum)	Yes
Female genital tract (vagina, uterus)	Yes
Occlusive hydrocephalus	ND
Impaired fertility	ND
Impaired lactation	Yes
Wasting	Yes
Impaired learning	No
Increased mortality	Yes
Impaired tissue repair	
Intravascular thrombosis	Yes
Skin (incisional wounds)	Yes
Tympanic membrane (rupture)	ND
Cornea (excimer laser ablation, scrape)	Yes
Kidney (glomerulonephritis)	ND
Joints (antigen-induced arthritis)	Yes
Skeletal muscle (crush injury)	Yes
Heart (myocardial infarction)	ND
Peripheral nervous system (crush injury)	Yes
Liver (necrosis)	No
Central nervous system (kainite-induced seizure, amyloid deposition)	No

[a]Fibrin and inflammatory cell-rich white, yellowish or reddish pseudomembranous, occasionally vascularized lesions named after their wood-like (ligneous) appearance

[b]Phenotype alleviated by fibrinogen deficiency, fibrinogen haploinsufficiency, or fibrinogen depletion

ND Not determined

Compiled from Akassoglou et al. (2000, 2002), Baykul and Bozkurt (2004), Bezerra et al. (1999), Bugge et al. (1995a, 1996b), Busso et al. (1998), Chambers et al. (1969), Ciftci et al. (2003), Cooper et al. (1979), Creemers et al. (2000), Drew et al. (1998), Eagle et al. (1986), Eriksson et al.

Fibrinogen expression is neglectable within the central nervous system under nonpathological conditions, and plasmin exerts its critical functions in brain homeostasis, injury repair, and learning independent of fibrin cleavage. In this regard, a number of candidate plasmin substrates have been identified, including probrain-derived neurotropic factor, laminin, proteoglycans, and amyloid-β. This indicates that plasminogen has multiple proteolytic targets in the brain (Melchor, Pawlak and Strickland 2003, Nakagami et al. 2000, Pang et al. 2004, Wu et al. 2000).

Physiological Functions of Inhibitors of Plasmin and Plasminogen Activators

α_2-Antiplasmin

Congenital autosomal α_2-antiplasmin deficiency is a rare disorder in humans that was first described in 1978 (Koie et al. 1978). Affected individuals are normal at birth, but suffer a moderate to severe lifelong predisposition for spontaneous and trauma-induced bleeding, as well as rebleeding after hemostasis has been achieved (Table 10.2). Episodes reported in these individuals include umbilical cord bleeding, urinary tract bleeding, bleeding gums, bleeding into the chest cavity, joint bleeding, spontaneous and traumatic subcutaneous bleeding, subarachnoid, epidural, and cerebral bleeding, and excessive bleeding after tooth extraction (Harish et al. 2006, Kluft et al. 1979, Kluft et al. 1982, Miles et al. 1982, Yoshinaga et al. 2000, Yoshioka et al. 1982). These bleeding episodes appear to be secondary to accelerated fibrinolysis, and they have been effectively treated with the plasmin inhibitor tranexamic acid, which inhibits the binding of plasminogen to fibrin (Kettle and Mayne 1985, Kluft et al. 1982, Yoshioka et al. 1982). The underlying cause of congenital α_2-antiplasmin deficiency, where determined, was attributed to splice site mutations, frameshift mutations, in frame deletions, and missense mutations within the α_2-antiplasmin gene (Hanss et al. 2003, Holmes et al. 1987, Lind and Thorsen 1999, Miura et al. 1989a, Miura et al. 1989b, Yoshinaga et al. 2000). Interestingly, relatives of affected individuals with heterozygous α_2-antiplasmin deficiency display a mild bleeding tendency (Hanss et al. 2003, Kluft et al. 1982, Leebeek et al. 1988, Miles et al. 1982).

Unlike humans, α_2-antiplasmin deficiency in mice does not appear to be associated with spontaneous bleeding under standard animal housing conditions.

Table 10.2 (Continued) (2006), Green et al. (2006), Gunhan et al. (1994), Hidayat and Riddle (1987), Holland et al. (1989), Hoover-Plow et al. (2001), Kao et al. (1998), Kitching et al. (1997), Kraft et al. (2000), Li et al. (2006), Lijnen et al. (1996), Lund et al. (1999, 2000), Mataga et al. (2004), Melchor et al. (2003), Mingers et al. (1997), Mizutani et al. (1996), Nakagami et al. (2000), Ng et al. (2001), Oray et al. (2004), Ozcelik et al. (2001), Pang et al. (2004), Pantanowitz et al. (2004), Ploplis et al. (1995), Pohl et al. (2001), Romer et al. (1996), Schott et al. (1998), Schuster et al. (1997, 1999b), Siconolfi and Seeds (2001), Suelves et al. (2002), Tabbara (2004), Tefs et al. (2006), Watts et al. (2002), and Wu et al. (2000)

Table 10.2 Physiological effects of a_2-antiplasmin-deficiency[a] in humans and mice

Humans
Spontaneous and trauma-induced bleeding episodes
Umbilical cord
Urinary tract
Gums
Chest cavity
Joints
Subcutaneous
Subarachnoid
Epidural
Cerebral
Tooth extraction socket
Mice[b]
Accelerated tissue repair
Intravascular thrombosis (endotoxin induced) Skin (incisional wounds)
Liver (necrosis)

[a]Congenital α_2-antiplasmin deficiency (heterozygous or homozygous deficiency)
[b]Spontaneous or trauma-induced bleeding episodes have not been reported in α_2-antiplasmin-deficient mice
Compiled from Hanss et al. (2003), Harish et al. (2006), Holmes et al. (1987), Kanno et al. (2006), Kettle and Mayne (1985), Kluft et al. (1979), Kluft et al. (1982), Koie et al. (1978), Leebeek et al. (1988), Lijnen et al. (1999), Lind and Thorsen (1999), Miles et al. (1982), Miura et al. (1989a), Miura et al. (1989b), Okada et al. (2004), Yoshinaga et al. (2000), and Yoshioka et al. (1982)

Furthermore, although lysis of fibrin clots was accelerated in these mice, bleeding times were not increased after tail tip or toe amputation (Lijnen et al. 1999). Interestingly, the increased plasmin activity caused by α_2-antiplasmin deficiency provided increased protection from endotoxin-induced thrombosis (Lijnen et al., 1999), accelerated the regeneration after toxic liver injuries, and enhanced skin wound healing (Kanno et al. 2006, Okada et al. 2004).

PAI-1

Humans with very low or undetectable PAI-1 have been identified (Dieval et al. 1991; Fay et al. 1997, 1992; Lee et al. 1993; Minowa et al. 1999; Schleef et al. 1989; Takahashi et al. 1996). In most cases, the molecular deficiency that underlies this autosomal recessive disorder has not been determined. However, thorough analysis of one large kindred, in which PAI-1 deficiency was frequent, uncovered a frameshift mutation in exon 4 of the PAI-1 gene, which leads to the generation of a null allele. Homozygosity for this null allele was documented in seven people and heterozygosity for the null allele was documented in 19 relatives of these inviduals (Fay et al. 1997, 1992). The collective analysis of PAI-1-deficient humans has revealed a critical role of PAI-1 in hemostasis (Table 10.3). PAI-1-deficient humans display supra-physiological levels of plasminogen activator activity, which causes a lifelong predisposition for spontaneous and trauma-induced bleeding. Reported

Table 10.3 Physiological effects of congenital PAI-1 deficiency in humans and mice

Humans
Spontaneous and trauma-induced bleeding episodes
Joints
Periosteum
Epidural
Tooth extraction socket
Chest cavity
Subcutaneous
Prolonged menstrual bleeding
Mice[a]
Accelerated tissue repair
Intravascular thrombosis (endotoxin induced)
Skin (incisional wounds)
Joints (antigen-induced arthritis)
Lungs (bleomycin-induced fibrosis)

[a] Spontaneous or trauma-induced bleeding episodes have not been reported in PAI-1-deficient mice
Compiled from Carmeliet et al. (1993a, 1993b), Chan et al. (2001), Dieval et al. (1991), Eitzman et al. (1996), Fay et al. (1997, 1992), Kawasaki et al. (2000), Lee et al. (1993), Minowa et al. (1999), Oda et al. (2001), Schleef et al. (1989), Suelves et al. (2005), Takahashi et al. (1996), Van Ness et al. (2002), and Zhu et al. (1999)

cases include recurrent bleeding into knee and elbow joints, subperiosteal bleeding after jaw trauma, epidural bleeding after head trauma, delayed bleeding after inguinal hernia surgery, prolonged bleeding after tooth extraction, and frequent bruising. Excessive menstrual bleeding is also a common predilection of PAI-1-deficient females. Bleeding episodes in PAI-1 deficient humans have been treated effectively by oral administration of α-aminocaproic acid or tranexamic acid (Dieval et al. 1991, Fay et al. 1997, 1992; Lee et al. 1993; Minowa et al. 1999; Schleef et al. 1989; Takahashi et al. 1996). Heterozygous siblings and parents of affected individuals were unremarkable. Given the many critical functions proposed for PAI-1 in cell migration, cell adhesion, angiogenesis, and immunity, it is curious that detailed physical examinations of humans with complete PAI-1-deficiency have failed to uncover any physiological abnormalities besides excessive spontaneous or trauma-induced bleeding.

PAI-1-deficient mice develop and reproduce normally, but present a hyperfibrinolytic state characterized by accelerated lysis of intravascular and ex vivo fibrin clots. However, like α_2-antiplasmin-deficient mice, the physiological consequences of loss of PAI-1 in mice appear to be less severe than in humans. Spontaneous bleeding episodes were not recorded in PAI-1-deficient mice under standard housing conditions, and the mice did not display increased bleeding or rebleeding after partial amputation of the tail or cecum (Carmeliet et al. 1993a, 1993b). However, consistent with the general impairment of tissue repair observed in mice with reduced plasminogen activation (plasminogen-deficient mice, plasminogen activator-deficient mice), the surpraphysiological state of activation of plasminogen

that accompanies PAI-1 deficiency appears to improve the time to healing and the overall outcome of a diverse number of tissue injuries in mice. These include intravascular thrombosis (Carmeliet et al. 1993b, Kawasaki et al. 2000, Zhu et al. 1999), obstructive kidney damage (Oda et al. 2001), skeletal muscle injury (Suelves et al. 2005), incisional skin wound healing (Chan et al. 2001), antigen-induced arthritis (Van Ness et al. 2002), and bleomycin-induced lung injury (Eitzman et al. 1996). The collective findings from these studies have made PAI-1 an increasingly attractive drug candidate.

Conclusions

Two decades of exhaustive analysis of humans and mice with genetic deficiencies in plasminogen, plasminogen activators, plasmin and plasminogen activator inhibitors have yielded fundamental new insights into the physiological role of the activation of plasminogen. Plasminogen and the components that govern plasminogen activation are dispensable for development. However, the cleavage of fibrin and other extracellular substrates by plasmin is critical to the postnatal remodeling and repair of multiple epithelial and mesenchymal tissues, and life without plasminogen is associated with high morbidity and mortality. At least five different pathways for the activation of plasminogen are operative in vivo, and the five pathways display a remarkable functional redundancy. Genetic deficiencies in inhibitors of plasmin and plasminogen activators not only cause lifelong bleeding predispositions but also accelerate tissue repair and regeneration.

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