Novel Functions of the Anticoagulant Activated Protein C in Maintaining Skin Barrier Integrity to Impact on Skin Disease

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Key Words

Activated protein C · Endothelial protein C receptor · Keratinocyte · Proliferation/migration · Junction proteins · Barrier function

Abstract

The epidermis is the outermost skin layer and provides the first line of defence against the external environment. Keratinocytes are the most predominant cells in the epidermis and play a critical role in maintaining epidermal barrier function. When the barrier is disrupted any of a number of diseases, such as chronic wounds, psoriasis, pemphigus, atopic dermatitis or toxic epidermal necrolysis, can take hold. Activated protein C (APC) or its precursor, protein C, is abundantly expressed by skin epidermal keratinocytes and stimulates their proliferation and migration, and inhibits apoptosis and inflammation, leading to a healing phenotype. Importantly, APC also increases the barrier function of keratinocytes by promoting expression and cell-cell contact redistribution of tight junction proteins. These cytoprotective properties of APC on epidermal keratinocytes place it as an exciting new therapy for skin disorders associated with the disruption of barrier function and inflammation.

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Skin Barrier Function

The skin forms an effective barrier against unwanted environmental insults such as mechanical trauma, pathogens, radiation, temperature fluctuations and excessive water loss from the body. Skin consists of two main layers, the epidermis, which is the outermost layer, and the underlying dermis, which provides the epidermis with mechanical support and nutrients. The barrier function of skin is mainly provided by the epidermis. At the cellular level, keratinocytes, the most abundant cell type in the epidermis, are responsible for maintaining the structure and homeostasis of this barrier. The epidermal barrier is generated by a sophisticated differentiation process composed of the stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS) and stratum basale (SB) [1] (fig. 1). Each layer displays one of the sequential differentiation stages of the keratinocytes, which comprise ~95% of the cells in the epidermis. The SB consists of proliferating keratinocytes, which maintain the epidermis and postmitotic basal keratinocytes that migrate out of the SB. After the keratinocytes escape from the SB they transiently migrate towards the SC. Eventually, keratinocytes end their lives in the SC and are sloughed off, a process called desquamation. The epidermis has a complete self-renew-

Fig. 1. General structure of the skin epidermis.

Fig. 2. Schematic representation of the basic components of tight junctions.

al capacity with an estimated turnover time of approximately 40 days in humans [2].

The barrier function of epidermis is primarily mediated by keratinocytes that forms a physical barrier and is supported by both adaptive and innate arms of the immune system. The physical barrier of the epidermis is localised primarily in the SC. The SC consists of keratinocytes that have undergone terminal differentiation, termed corneocytes, with degradation of the nucleus, loss of DNA and formation of a unique cornified envelope. This layer prevents the movement of water and electrolytes through the SC that is essential for life. SC also plays

an important role in the barrier to infection. The underlying nucleated epidermis which contains live keratinocytes, with its tight, gap and adherens junctions, additionally contributes to the barrier [3–5]. These proteins seal the intercellular space between cells and control the movement of molecules. The tight junctions comprise the extracellular proteins, occludins, junctional adhesion molecule (JAM), claudins and Tie2, and intracellular proteins, such as ZO-1/2/3 (fig. 2). Deregulation of these junction proteins perturbs the skin barrier [3, 4]. For example, deficiency of claudin-1 results in epidermal water loss and, ultimately, the neonatal death of mice [6]. The immunological barrier of the epidermis is provided by Langerhans cells in the SS, melanocytes and keratinocytes. The latter act via their capacity to produce mucus, antimicrobial peptides, metabolites, UV-absorbing molecules and Toll receptors, and cytokines/chemokines play important roles both in the skin's adaptive and innate defences [7].

Skin Diseases Associated with Barrier Disruption

A defective epidermal barrier is observed in many inflammatory and blistering skin disorders, such as atopic dermatitis, pemphigus, psoriasis, chronic wounds, and the devastating and often fatal toxic epidermal necrolysis. [3, 8, 9]. Atopic dermatitis affects 15–30% of children and 2–10% of adults, and its prevalence has increased by 2- to 3-fold during the past 3 decades, especially in developed countries [10]. Psoriasis is a chronic inflammatory skin disease affecting about 2% of the population of Western countries. The disease is characterised by abnormal epidermal proliferation leading to an incomplete differentiation of keratinocytes and impaired barrier function [11]. Chronic wounds affect around 1% of the population. In contrast to physiologically healing wounds, chronic wounds fail to restore the barrier function of skin. Often a persistence of the inflammatory phase is responsible for wound chronicity [12].

Activated Protein C

Activated protein C (APC) is formed when circulating protein C (PC) is activated by thrombin bound to thrombomodulin on the endothelial cell surface. The conversion is augmented by PC binding to endothelial cell PC receptor (EPCR) [13]. In the presence of its cofactor, pro-

tein S, APC regulates blood coagulation by degrading the coagulation factors Va and VIIIa to prevent thrombin generation. In addition, APC promotes fibrinolysis by binding to plasminogen activator inhibitor to prevent the conversion of plasminogen to plasmin. The importance of APC as an anticoagulant is reflected by findings that deficiencies in PC result in severe familial disorders of thrombosis [14]. Replenishment of PC in patients with systemic or local hypercoagulation can reverse the abnormality.

Independent of its anticoagulant activity, APC possesses strong anti-inflammatory, anti-apoptotic and barrier-stabilising properties, which are protective in many autoimmune and inflammatory diseases, including sepsis, diabetes, spinal cord injury and asthma [15]. The cytoprotective properties of APC are mainly mediated through EPCR, protease-activated receptor (PAR)-1 or epidermal growth factor receptor (EGFR) [16, 17]. Recent studies show that APC stabilises the cytoskeleton and reduces endothelial permeability to enhance the integrity of blood vessels by acting through PAR-1 and Tie2 [18]. In keratinocytes, APC acts by binding to EPCR, cleaving PAR-1 and transactivating EGFR, followed by transactivation of Tie2 to promote barrier function [17]. APC can also utilise PAR-2 in mice to promote excisional wound healing [19].

Protective Effects of APC in Skin

PC/APC Is Expressed in the Epidermis

In 2007, we first demonstrated that human epidermis and cultured human keratinocytes strongly express PC and APC [20]. These cells also express all other members of the PC pathway, including EPCR, thrombomodulin, thrombin, PC inhibitor, PAR-1, PAR-2, EGFR and Tie2 [21]. That is, the epidermis possesses its own independent PC system which can synthesize PC, convert PC to its active form (i.e. APC), regulate the activity of APC and mediate its function [20].

In vitro, the removal of endogenous PC or APC with siRNA or blocking antibodies, respectively, results in inhibition of the normal function of keratinocytes, with an increase in apoptosis and loss of barrier function. In mice, total PC knockout causes lethal perinatal consumptive coagulopathy soon after birth [22]. Mice with 1% of the normal PC level survive but are characterised by thrombosis, inflammation and haemorrhagic skin lesions [23]. PC deficiency in humans manifests in a variety of cutaneous signs, such as ecchymoses and rapidly progressive ne-

crosis of the skin, as occurs in purpura fulminans [24–27]. Homozygous PC deficiency often results in neonatal purpura fulminans and is usually fatal [28].

APC Stimulates Keratinocytes

APC promotes the growth of various cultured cells, including endothelial cells, smooth muscle cells, keratinocytes, neural stem and progenitor cells, neuroblasts, osteoblasts and tenocytes via modulation of MAP kinase activity [15, 29]. Consistent with the stimulatory effects on cell growth, APC displays strong anti-apoptotic properties, both in vitro and in vivo, via the inhibition of pro-apoptotic factors, caspase-3, 8, 9 [30] and p53 [31], and upregulation of anti-apoptotic molecules, including endothelial nitric oxide synthase and Bcl-2 homologue [32].

Keratinocyte proliferation and migration is key for the normal turnover of epidermis and to restore its function after injury by replacing the lost tissue. In cultured human skin keratinocytes, exogenous APC promotes proliferation, which is mediated by EPCR, PAR-1 and EGFR, and subsequent selective activation of MAP kinases [33]. Consistent with the stimulatory effects on cell growth, APC prevents apoptosis of keratinocytes via inhibition of pro-apoptotic factors, and the induction of anti-apoptotic mediators. APC suppresses the activation of the apoptotic marker, caspase-3, in cultured keratinocytes and caspase-3 activation is increased when PC is suppressed by siRNA, which is consistent with a role for PC in preventing keratinocyte apoptosis [33].

APC promotes the migration of keratinocytes in a concentration-dependent manner [34], while inhibition of endogenous PC reduces keratinocyte migration in vitro [33]. This function of APC may be partly mediated via regulation of matrix metalloproteinases (MMPs), a family of structurally related zinc- and calcium-dependent endopeptidases capable of degrading extracellular components. Blockade of MMPs using GM6001, a broad spectrum MMP inhibitor, eliminates cell migration in a dose-dependent manner and delays in vitro wound healing [34]. In vivo, MMP-2 is constitutively expressed in normal skin and epidermis, whilst the expression of MMP-9 is almost always associated with inflammatory conditions, such as psoriasis and chronic wounds [35]. In culture, human keratinocytes produce both MMP-2 and MMP-9 [36]. Agren et al. [37] found that whereas MMP-2 is required for keratinocyte migration, MMP-9 is not necessary. APC stimulates and activates MMP-2, which also has anti-inflammatory properties, while having either no effect on or inhibiting MMP-9 (fig. 3a).

Fig. 3. The effect of APC on cultured human keratinocytes. **a** MMP-2 and MMP-9 in cultured supernatants of human keratinocyte monolayers in response to APC or TNF for 24 h, detected by zymography. **b** Tie2 and ZO-1 expression and redistribution in keratinocytes in response to 1 μg/ml of APC for 1 h, detected by immunofluorescent staining. Scale bars = 20 μm.

In vivo, keratinocyte proliferation and migration are required for re-epithelialization during wound healing as it serves to restore the barrier function of skin. In full-thickness excisional skin wound healing models of rat or mouse, topical application of APC enhances wound healing by stimulating re-epithelialization [19, 38].

APC Promotes Skin Barrier Function

Endothelial cells normally form a dynamically regulated stable barrier at the blood-tissue interface, which controls the transfer of molecules and leukocytes into and out of the bloodstream. Breakdown of this barrier is a key pathogenic factor in many inflammatory diseases, including cancer, sepsis, rheumatoid arthritis and diabetes. APC enhances endothelial barrier integrity by stabilising/rearranging the cytoskeleton, promoting tight junction protein expression and cell-cell contact redistribution [39]. Barrier stabilisation is more effective when APC is derived endogenously and functions in an autocrine manner than when the source of APC is exogenous [40]. Recent studies have demonstrated a new role of the PC system in controlling epithelial permeability, for example by regulating tight junction molecules to reduce intestinal permeability and promote mucosal healing

[41]. The effect of APC on the skin epithelium barrier is discussed below.

In cultured keratinocyte monolayers, APC decreases paracellular permeability in a dose-dependent manner by upregulating tight junction proteins and redistributing them to cell-cell contacts [17]. In response to APC treatment, the junctional proteins Tie2/phosphorylated Tie2 and ZO-1 relocate to cell-cell contacts within 1 h where they impede barrier permeability (fig. 3b) [17]. Expression of ZO-1, claudin-1 and vascular endothelial cadherin, three important proteins that maintain the epidermal barrier, is subsequently increased. Interestingly, APC does not activate Tie2 through its major ligand, angio-poeitin-1, but binds directly to EPCR, cleaves PAR-1 and transactivates EGFR, then Tie2 activates PI3K/Akt signalling to stimulate junctional complexes and reduce keratinocyte permeability [17].

In severe PC-deficient mice, lack of PC leads to decreased JAM-A and claudin-3 expression, and to an altered pattern of ZO-1 expression in the epithelium [41]. In addition, APC reduces the thrombin-induced disruption of alveolar epithelial barrier integrity via decreasing epithelial permeability, cell stiffening, cell contraction and enhancing ZO-1 aggregates at the cell-cell interface [42].

APC Contributes to the Immunological Barrier of the Epidermis

The anti-inflammatory effects of APC are associated with a decrease in pro-inflammatory cytokines and a reduction in leukocyte recruitment. APC inhibits neutrophil, monocyte and lymphocyte migration and invasion, and directly suppresses the expression and activation of inflammatory signalling molecules nuclear factor (NF)- κ B, AP-1, and inflammatory mediators such as tumour necrosis factor (TNF)- α [43]. During acute inflammation, plasma APC levels are diminished [44]. A thrombomodulin mutation that impairs APC generation results in uncontrolled lung inflammation during murine tuberculosis [45]. Acute inflammation is exacerbated in mice genetically predisposed to a severe PC deficiency [46]. The level of PC strongly correlates with survival outcomes following endotoxin challenge in low-PC mice and administration of recombinant human APC extends the survival of these animals [47].

In culture, APC inhibits inflammatory mediator production by keratinocytes [33, 34]. The NF- κ B pathway is important for expression of a wide variety of inflammatory genes, including TNF- α and cell adhesion molecules. APC inhibits calcium- and lipopolysaccharide-stimulated activation of NF- κ B in keratinocytes [34].

Prospective Therapeutic Potential of PC/APC in Skin Diseases

The skin, the body's largest organ, provides an epidermal barrier to protect the body from external insults, maintain temperature and control evaporation. Breaches of this barrier can result in many skin diseases. These skin-associated diseases are common and affect approximately 50% of people worldwide at any given time. The burden of skin disease encompasses psychological, social and financial consequences on patients, their families and on society. Total direct expenditure in the National Health Service (NHS) in England and Wales in 2005/2006 for skin diseases was GBP 1,424 million, representing 2.23% of total NHS expenditure [48].

There is no cure for skin diseases such as psoriasis and atopic dermatitis; topical medications, phototherapy, traditional systemic agents and biologics only offer options for the management of symptoms. A combination of agents is frequently needed for moderate-to-severe cases and positive long-term outcomes require medication adherence [49]. For other conditions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, no treat-

Fig. 4. The function of APC on epidermal keratinocytes.

ment has been identified to date that is capable of halting the progression of skin detachment.

Although APC efficacy and safety is controversial in the treatment of sepsis patients, it has emerged as a potential treatment for skin diseases by stimulating re-epithelisation, suppressing inflammation and stabilising barrier function. In animal models, APC promotes cutaneous healing of full-thickness punch biopsy wounds in rats and mice by stimulating re-epithelialization and by inhibition of inflammation [19, 38]. Recent evidence demonstrates that APC is also effective in human trials involving chronic wounds of venous and diabetic origin [50], recalcitrant orthopaedic wounds [51], pressure sores [52] and ulcers associated with pyoderma gangrenosum [48].

Conclusions

The protective barrier function of APC provides direction for new and better strategies to treat the myriad of skin diseases affected by abnormal epidermal barrier function and epidermal regeneration. Figure 4 summarises the actions of APC on skin epidermal keratinocytes. The future for utilising exogenous APC as a topical treatment for skin inflammatory conditions remains a novel and exciting avenue of investigation.

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Disclosure Statement

The authors have granted and pending patents on this topic and are shareholders in a related company.

References

- Kalinin A, Marekov LN, Steinert PM: Assembly of the epidermal cornified cell envelope. *J Cell Sci* 2001;114:3069–3070.
- Weinstein GD, McCullough JL, Ross P: Cell proliferation in normal epidermis. *J Invest Dermatol* 1984;82:623–628.
- Proksch E, Brandner JM, Jensen JM: The skin: an indispensable barrier. *Exp Dermatol* 2008;17:1063–1072.
- Proksch E, Brasch J: Abnormal epidermal barrier in the pathogenesis of contact dermatitis. *Clin Dermatol* 2012;30:335–344.
- Vandenbroucke E, Mehta D, Minshall R, Malik AB: Regulation of endothelial junctional permeability. *Ann NY Acad Sci* 2008;1123:134–145.
- Furuse M, Hata M, Furuse K, Yoshida Y, Haraake A, Sugitani Y, Noda T, Kubo A, Tsukita S: Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002;156:1099–1111.
- Lee SH, Jeong SK, Ahn SK: An update of the defensive barrier function of skin. *Yons Med J* 2006;47:293–306.
- Niyonsaba F, Ogawa H: Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents β -defensins, cathelicidin LL-37 and lysozyme. *J Dermatol Sci* 2005;40:157–168.
- Roberson ED, Bowcock AM: Psoriasis genetics: breaking the barrier. *Trends Genet* 2010;26:415–423.
- Leung DY, Bieber T: Atopic dermatitis. *Lancet* 2003;361:151–160.
- Albanesi C, De Pita O, Girolomoni G: Resident skin cells in psoriasis: a special look at the pathogenetic functions of keratinocytes. *Clin Dermatol* 2007;25:581–588.
- Gurtner GC, Werner S, Barrandon Y, Longaker MT: Wound repair and regeneration. *Nature* 2008;453:314–321.
- Fukudome K, Esmon CT: Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem* 1994;269:26486–26491.
- Baker WF Jr, Bick RL: Treatment of hereditary and acquired thrombophilic disorders. *Semin Thromb Hemost* 1999;25:387–406.
- Jackson C, Whitmont K, Tritton S, March L, Sambrook P, Xue M: New therapeutic applications for the anticoagulant, activated protein C. *Expert Opin Biol Ther* 2008;8:1109–1122.
- Esmon CT: Structure and functions of the endothelial cell protein C receptor. *Crit Care Med* 2004;32:S298–S301.
- Xue M, Chow SO, Dervish S, Chan YK, Julovi SM, Jackson CJ: Activated protein C enhances human keratinocyte barrier integrity via sequential activation of epidermal growth factor receptor and Tie2. *J Biol Chem* 2011;286:6742–6750.
- Minhas N, Xue M, Fukudome K, Jackson CJ: Activated protein C utilizes the angiopoietin/Tie2 axis to promote endothelial barrier function. *FASEB J* 2010;24:873–881.
- Julovi SM, Xue M, Dervish S, Sambrook PN, March L, Jackson CJ: Protease activated receptor-2 mediates activated protein C-induced cutaneous wound healing via inhibition of p38. *Am J Pathol* 2011;179:2233–2242.
- Xue M, March L, Sambrook PN, Fukudome F, Jackson CJ: Endothelial protein C receptor is over-expressed in rheumatoid arthritic (RA) synovium and mediates the anti-inflammatory effects of activated protein C in RA monocytes. *Ann Rheum Dis* 2007;66:1574–1580.
- McKelvey K, Jackson CJ, Xue M: Activated protein C: a regulator of human skin epidermal keratinocyte function. *World J Biol Chem* 2014;5:169–179.
- Jalbert LR, Rosen ED, Moons L, Chan JC, Carmeliet P, Collen D, Castellino FJ: Inactivation of the gene for anticoagulant protein C causes lethal perinatal consumptive coagulopathy in mice. *J Clin Invest* 1998;102:1481–1488.
- Lay AJ, Liang Z, Rosen ED, Castellino FJ: Mice with a severe deficiency in protein C display prothrombotic and proinflammatory phenotypes and compromised maternal reproductive capabilities. *J Clin Invest* 2005;115:1552–1561.
- Salonvaara M, Kuismanen K, Mononen T, Riikonen P: Diagnosis and treatment of a newborn with homozygous protein C deficiency. *Acta Paediatr* 2004;93:137–139.
- Parsi K, Younger I, Gallo J: Warfarin-induced skin necrosis associated with acquired protein C deficiency. *Australas J Dermatol* 2003;44:57–61.
- Agras PI, Ozdemir H, Baskin E, Ozbek N: Recurrent vasculopathic skin lesions associated with homozygous protein C deficiency. *Pediatr Dermatol* 2007;24:57–60.
- Boyvat A, Kundakci N, Babikir MO, Gurgey E: Livedoid vasculopathy associated with heterozygous protein C deficiency. *Br J Dermatol* 2000;143:840–842.
- Price VE, Ledingham DL, Krumpel A, Chan AK: Diagnosis and management of neonatal purpura fulminans. *Semin Fetal Neonatal Med* 2011;16:318–322.
- Xue M, Jackson CJ: Activated protein C and its potential applications in prevention of islet β -cell damage and diabetes. *Vitam Horm* 2014;95:323–363.
- Du ZJ, Yamamoto T, Ueda T, Suzuki M, Tano Y, Kamei M: Activated protein C rescues the retina from ischemia-induced cell death. *Invest Ophthalmol Vis Sci* 2011;52:987–993.
- Cheng T, Liu D, Griffin JH, Fernandez JA, Castellino F, Rosen ED, Fukudome K, Zlokovic BV: Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med* 2003;9:338–342.
- Isermann B, Vinnikov IA, Madhusudhan T, Herzog S, Kashif M, Blautzik J, Corat MAF, Zeier M, Blessing E, Oh J, Gerlitz B, Berg DT, Grinnell BW, Chavakis T, Esmon CT, Weiler H, Bierhaus A, Nawroth PP: Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat Med* 2007;13:1349–1358.
- Xue M, Campbell D, Jackson CJ: Protein C is an autocrine growth factor for human skin keratinocytes. *J Biol Chem* 2007;282:13610–13616.
- Xue M, Thompson P, Kelso I, Jackson C: Activated protein C stimulates proliferation, migration and wound closure, inhibits apoptosis and upregulates MMP-2 activity in cultured human keratinocytes. *Exp Cell Res* 2004;299:119–127.
- Oikarinen A, Kylmaniemi M, Autio-Harmanen H, Autio P, Salo T: Demonstration of 72-kDa and 92-kDa forms of type IV collagenase in human skin: variable expression in various blistering diseases, induction during re-epithelialization, and decrease by topical glucocorticoids. *J Invest Dermatol* 1993;101:205–210.
- Xue M, Jackson CJ: Autocrine actions of matrix metalloproteinase (MMP)-2 counter the effects of MMP-9 to promote survival and prevent terminal differentiation of cultured human keratinocytes. *J Invest Dermatol* 2008;128:2676–2685.
- Agren MS, Mirastschijski U, Karlsmark T, Saarialho-Kere UK: Topical synthetic inhibitor of matrix metalloproteinases delays epidermal regeneration of human wounds. *Exp Dermatol* 2001;10:337–348.

- 38 Jackson CJ, Xue M, Thompson P, Davey RA, Whitmont K, Smith S, Buisson-Legendre N, Sztynka T, Furphy LJ, Cooper A, Sambrook P, March L: Activated protein C prevents inflammation yet stimulates angiogenesis to promote cutaneous wound healing. *Wound Repair Regen* 2005;13:284–294.
- 39 Bouwens EA, Stavenhagen F, Mosnier LO: Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway. *J Thromb Haemost* 2013;11(suppl 1):242–253.
- 40 Feistritzer C, Schuepbach RA, Mosnier LO, Bush LA, Di CE, Griffin JH, Riewald M: Protective signaling by activated protein C is mechanistically linked to protein C activation on endothelial cells. *J Biol Chem* 2006;281:20077–20084.
- 41 Vetrano S, Ploplis VA, Sala E, Sandoval-Cooper M, Donahue DL, Correale C, Arena V, Spinelli A, Repici A, Malesci A, Castellino FJ, Danese S: Unexpected role of anticoagulant protein C in controlling epithelial barrier integrity and intestinal inflammation. *Proc Natl Acad Sci USA* 2011;108:19830–19835.
- 42 Puig F, Fuster G, Adda M, Blanch L, Farre R, Navajas D, Artigas A: Barrier-protective effects of activated protein C in human alveolar epithelial cells. *PLoS One* 2013;8:e56965.
- 43 Esmon CT: Crosstalk between inflammation and thrombosis. *Maturitas* 2004;47:305–314.
- 44 Liaw PC, Esmon CT, Kahnamouy K, Schmidt S, Kahnamouy S, Ferrell G, Beaudin S, Julian JA, Weitz JI, Crowther M, Loeb M, Cook DJ: Patients with severe sepsis vary markedly in their ability to generate activated protein C. *Blood* 2004;104:3958–3964.
- 45 Weijer S, Wieland CW, Florquin S, van der Poll T: A thrombomodulin mutation that impairs activated protein C generation results in uncontrolled lung inflammation during murine tuberculosis. *Blood* 2005;106:2761–2768.
- 46 Lay AJ, Donahue D, Tsai MJ, Castellino FJ: Acute inflammation is exacerbated in mice genetically predisposed to a severe protein C deficiency. *Blood* 2007;109:1984–1991.
- 47 Xu J, Ji Y, Zhang X, Drake M, Esmon CT: Endogenous activated protein C signaling is critical to protection of mice from lipopolysaccharide-induced septic shock. *J Thromb Haemost* 2009;9:851–856.
- 48 Kapila S, Reid I, Dixit S, Fulcher G, March L, Jackson C, Cooper A: The use of dermal injection of activated protein C for treatment of large chronic wounds secondary to pyoderma gangrenosum. *Clin Exp Dermatol* 2014;39:785–790.
- 49 Zanni GR: Psoriasis: issues far more serious than cosmetic. *Consult Pharm* 2012;27:86–88, 90, 93–86.
- 50 Whitmont K, Reid I, Tritton S, March L, Xue M, Lee M, Fulcher G, Sambrook P, Slobedman E, Cooper A, Jackson C: Treatment of chronic leg ulcers with topical activated protein C. *Arch Dermatol* 2008;144:1479–1483.
- 51 Wijewardena A, Vandervord E, Lajevardi SS, Vandervord J, Jackson CJ: Combination of activated protein C and topical negative pressure rapidly regenerates granulation tissue over exposed bone to heal recalcitrant orthopedic wounds. *Int J Low Extrem Wounds* 2011;10:146–151.
- 52 Wijewardena A, Lajevardi SS, Vandervord E, Vandervord J, Lang TC, Fulcher G, Jackson CJ: Activated protein C to heal pressure ulcers. *Int Wound J* 2014, Epub ahead of print.