Therapeutic potential of human elafin

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Abstract

Elafin is an endogenous human protein composed of an N-terminal transglutaminase substrate motif and a C-terminal WAP (whey acidic protein)-domain with antiproteolytic properties. Elafin is expressed predominantly in epithelial tissue and potently inhibits the neutrophil-derived serine proteases elastase and proteinase-3 by a competitive tight-binding mechanism. Furthermore, it inhibits EVE (endogenous vascular elastase). Studies on several animal models show that antiprotease augmentation with human elafin is an effective strategy in the treatment of inflammatory vascular, systemic and pulmonary diseases and of inflammation triggered by reperfusion injury. This raises the possibility that elafin might be effective in the treatment of a variety of human inflammatory diseases. In a Phase I clinical trial, elafin was well tolerated. Phase II trials are underway to investigate the therapeutic effects of elafin on post-operative inflammation and the clinical consequences of major surgery. Of particular interest is the reduction of post-operative morbidity after oesophagus cancer surgery, coronary artery bypass surgery and kidney transplantation.

Introduction

The serine elastase inhibitor elafin is produced constitutively by epithelia as part of the body’s antiprotease shield, e.g. in the skin, upper gastrointestinal tract, female reproductive tract and lungs, and its expression is up-regulated by inflammatory stimuli [1–4]. Low levels of elafin are also found in the serum and increase significantly in the presence of inflammatory diseases, such as psoriasis [5] and acute respiratory distress syndrome [6].

Elafin was first isolated from scales of psoriatic skin [7,8] as a soluble cationic non-glycosylated protein with a molecular mass of 6 kDa. In epithelia, elafin is expressed as a 9.9 kDa protein, which consists of two functional domains. The N-terminal domain contains four transglutaminase substrate repeats and the C-terminal domain shows folding and arrangement of disulfide bonds characteristic of WAP (whey acidic proteins) [9].

Functionally, the N-terminal domain allows the transglutaminase-mediated immobilization of elafin to ECM (extracellular matrix) proteins [10] and the C-terminus harbours the protease inhibition domain. The protease inhibitory function is dominated by its potent and specific inhibition of the human neutrophil proteases elastase and proteinase-3 [7,11]. Elafin inhibits these serine proteases by a tight-binding reversible mechanism leading to an almost 1:1 molar inhibition (Kᵢ for human leucocyte elastase 0.43 nM and proteinase-3 0.42 nM). The inhibitory active centre is located in an exposed loop that is stabilized by four disulfide bonds of the WAP-domain core [9,12,13]. Elafin further inhibits EVE (endogenous vascular elastase), a serine protease produced by diseased vascular tissue [14,15].

Elafin naturally occurs as a full-length 9.9 kDa protein as well as in several N-terminally truncated forms, such as the soluble 6 kDa form which is the dominant form present in psoriatic skin. Several groups have worked with elafin and alternative names are in use. Elafin is predominantly used in protein biochemistry and pharmacology and forms the prototype of the family of elafin-like proteins in the SCOP (structural classification of proteins). In comparative biology, elafin is also referred to as trappin-2 [16]. Some authors discriminate between proelafin (9.9 kDa) and elafin (6 kDa) [17].

Elafin’s biological functions arise not only from its protease-inhibitory properties but also from other molecular properties and have an influence on cellular proliferation, inflammation and infections.

Biological properties of elafin

Cellular proliferation

Elastase is known to be present in an active form in the epidermis of psoriatic lesions in contrast with healthy or uninvolved skin [18]. It has been shown that elastase is able to induce pronounced epidermal thickening within 3 days after repeated topical application to mouse skin [19]. This can be abolished by the addition of elafin, indicating a dependence on the proteolytic activity. In keratinocyte cultures, the addition of elastase induces the release of TGFα (transforming growth factor α) from a keratinocyte membrane-bound form, followed by EGFR (epidermal growth factor receptor) activation. Elafin abolishes this elastase-induced keratinocyte activation and hyperproliferation [19,20].
In certain vascular diseases, e.g. PAH (pulmonary arterial hypertension) and arterial injury, EVE promotes matrix protein deposition and the proliferation of VSMCs (vascular smooth muscle cells), leading to intimal thickening and vessel occlusion. In animal models of pulmonary arterial hypertension [21] and arterial injury [22], human elafin significantly reduces vascular remodelling.

**Inflammation**

Neutrophil activation stimulates the transfer of elastase and proteinase-3 to phagosomes or their export to the plasma membrane from where they may be released into the ECM [23]. Elastase and proteinase-3 play the following roles in initiating and sustaining an inflammatory response: (i) degradation of ECM proteins [23]; (ii) combating pathogens by phagosomal degradation of bacterial proteins [24]; (iii) generation of chemotactic peptides [25]; (iv) activation of MMPs (matrix metalloproteinases) [26,27]; and (v) induction [28–30] and proteolytic activation [31,32] of pro-inflammatory cytokines.

Inhibition of elastase and proteinase-3 by elafin thus attenuates several key processes in the inflammatory cascade.

**Antibacterial properties**

In addition to protease inhibition, elafin also displays antibacterial properties [33] that are independent of its antiprotease activity. This may be due to the highly cationic nature of the molecule causing cell membrane disruption [34]. Furthermore, elafin inhibits the LPS (lipopolysaccharide)-stimulated monocyte chemotactic protein-1 formation by inhibiting AP-1 (activator protein 1) and NF-κB (nuclear factor κB) activation in monocytes. This occurs via an effect on the ubiquitin–proteasome pathway [35]. In the presence of serum, elafin binds LPS and suppresses its stimulation of macrophages to produce the pro-inflammatory cytokine TNFα (tumour necrosis factor α) [36].

**Antiviral activity**

Elafin/trappin-2 has been identified as a factor in female genital secretions, which correlates with resistance to HIV infection [37]. This antiviral effect requires a direct interaction between HIV and elafin [2].

**Lessons learned from elafin-overexpressing transgenic mice**

Rabinovitch and co-workers [38] have created transgenic mice which overexpress human elafin in the cardiovascular system as well as in kidneys, lungs and skin. With these mice, a set of experiments has been conducted providing evidence that elafin may be effective in treating myocardial ischaemia, viral inflammation and proliferative inflammatory vasculopathies.

**Myocardial ischaemia**

Anoxia of cardiac muscle during a myocardial infarction triggers inflammation causing impairment of cardiac function. A myocardial infarction was simulated by a permanent ligature on the left anterior descending coronary artery of elafin-overexpressing and control mice [39]. Compared with non-transgenic animals, elafin-overexpressing mice exhibited lower tissue elastase activity, reduced neutrophil infiltration of cardiac tissue, diminished left ventricular fibrosis and decreased infarct size.

**Viral myocarditis**

Intraperitoneal infection of non-transgenic mice with encephalomyocarditis virus causes cardiac muscle inflammation, followed by fibrosis and impaired myocardial function. Elafin-transgenic mice showed a pronounced reduction in inflammatory injury of the myocardium with retention of cardiac function and a marked reduction in mortality [38] as compared with the non-transgenic controls.

**Arterial injury**

Arterial injury triggers neutrophil infiltration and neointimal thickening by smooth muscle cell proliferation. Released neutrophil elastase and EVE promote this process by generating chemoattractant peptides and liberating growth factors. To test the effects of elafin on this pathology, the carotid artery of elafin transgenic mice and control animals was damaged using wire. Elafin-transgenic mice showed suppressed injury-induced appearance of elastase in the tissue, resulting in fewer proliferating smooth muscle cells and reduced neointima formation [22].

**Hypoxic pulmonary hypertension**

PAH is characterized by a progressive increase in pressure in the pulmonary artery with resulting right ventricular hypertrophy. In PAH, EVE and neutrophil elastase are up-regulated in affected tissues, leading to MMP activation and growth factor release. This promotes smooth muscle cell hyperproliferation and aberrant ECM deposition in pulmonary and distal pulmonary arteries, leading to their narrowing and restricted blood flow.

The effect of elafin on this pathology was investigated in elafin-transgenic mice using the hypoxia-induced PAH model. Compared with non-transgenic animals, elafin-expressing mice exhibited no increase in pulmonary elastase and reduced MMP-9 and MMP-2. In transgenic mice, pulmonary artery muscularization and the right ventricular pressure increase were significantly lower than in non-transgenic littermates [21].

**Lessons learned from elafin transfection**

The transient expression of human elafin was achieved by flushing the relevant tissue with haemagglutinating virus of Japan liposomes containing a plasmid encoding human elafin [40]. The studies, described below, indicate that elafin may be applicable in alleviating vasculopathies caused by vascular injury.
Vein graft degeneration
Vein grafts used in a coronary artery bypass often occlude due to intimal thickening and atherosclerosis. This is provoked by an inflammatory reaction elevating elastase activity with matrix degradation, growth factor release and a resulting proliferation and migration of smooth muscle cells. The effect of elafin on this process was studied in rabbits by transient elafin expression in excised jugular veins that were implanted into the carotid artery. Compared with mock-transfected grafts, elafin expression reduced inflammatory cell infiltration with diminished intimal and medial/adventitial thickening and atherosclerosis. The results showed that elafin expression ameliorates remodelling of implanted vein grafts, rendering them less susceptible to neointimal thickening and the development of atherosclerotic plaques [40].

Balloon angioplasty
Balloon angioplasty is used to improve blood flow in coronary arteries that have been narrowed by atherosclerosis. Despite a high success rate, long-term complications may arise, resulting in arterial injury, the deposition of ECM proteins, smooth muscle cell proliferation and neointimal hyperplasia. Using a rabbit model system, it was shown that balloon angioplasty of iliac arteries induced a transient increase in serine elastase activity. Neutrophil infiltration was modest, suggesting the involvement of EVE produced by smooth muscle cells. Elafin transfection in injured arterial segments lowered arterial elastase activity and reduced the intimal cross-sectional area [41].

Lessons learned from elafin administration
Elafin introduction by intravenous or pulmonary routes represents the most realistic mode of administration for clinical applications. The following animal studies demonstrate that intravenous elafin has the potential to alleviate ischaemia/reperfusion injury and associated arteriopathies. In addition, pulmonary administered elafin is a promising agent for the treatment of inflammation in the lung.

Heart transplantation in rabbits
Allograft coronary arteriopathy is a major complication in the survival of heart grafts. It is initiated by reperfusion injury that leads to endothelial cell activation and inflammatory cell recruitment. Elastase from invading inflammatory cells and EVE from smooth muscle cells catalyse the release or activation of pro-inflammatory cytokines, chemotactic factors and growth factors. These promote inflammation and VSMC proliferation and migration as well as the deposition of new ECM, resulting in progressive neointimal thickening and vessel occlusion. The potential of elafin in the alleviation of graft arteriopathy was tested using heterotopic cardiac transplantation in rabbits. Post-operative intravenous elafin resulted in reduced myocardial necrosis and attenuated coronary arteriopathy [15].

Hind limb ischaemia in rats
Reperfusion of ischaemic skeletal muscle initiates local and systemic inflammation. Elastase from invading neutrophils is a pathogenic factor in reperfusion injury and its inhibition might be effective in the treatment of this condition. To test this, unilateral hind limb ischaemia was induced in rats and followed by reperfusion [42]. Elafin was administered intravenously before and after reperfusion. Compared with control animals, elafin-treated muscle tissue exhibited less neutrophil infiltration and greater muscle viability after reperfusion.

Cardiac ischaemia in rats
The restoration of blood flow to the heart is essential in treating myocardial infarction. However, reperfusion injury of ischaemic cardiac muscle causes tissue damage with prolonged impairment of myocardial function. Elastase from infiltrating neutrophils is a pathological factor in reperfusion injury, suggesting that elafin administration might alleviate myocardial damage after an infarction. To study this, in situ perfused rat hearts were subjected either to repeated ischaemia and reperfusion or a simulated myocardial infarction. In both situations, intravenously administered elafin reduced neutrophil infiltration of cardiac muscle, leading to an improvement in myocardial function. After myocardial infarction elafin also decreased infarct size and the area at risk [43].

Potential target diseases
From the preclinical development, it is clear that the most consistent effects of elafin are in the suppression of neutrophil-mediated inflammatory tissue damage and vascular changes occurring during ischaemia. In the surgical field, these phenomena lead to severe disturbances which are associated with post-operative morbidity and mortality, and are particularly evident after major surgery, e.g. oesophagectomy, pancreaticoduodenectomy, major lung resection, gastrectomy and colorectal resection. As transthoracic oesophagectomy is associated with the most pronounced post-operative morbidity and mortality, it represents a potential indication for elafin treatment. Further potential indications are seen in the reduction of post-operative morbidity after coronary artery bypass surgery and kidney transplantation.

Transthoracic oesophagectomy with lymphadenectomy is performed for the treatment of oesophageal carcinoma and is one of the most invasive forms of surgery. The operation imposes considerable stress on the lungs, due to the need for deflation of one lung for surgical access to the carcinoma and one-sided artificial ventilation. This results in the activation of the numerous resident neutrophils with the release of elastase and induction of pro-inflammatory cytokines such as IL (interleukin)-6 and IL-8 [44]. This inflammatory reaction underlies the frequent post-operative pulmonary and systemic inflammatory complications associated with this
operation [45]. Peri-operative elastase inhibition by elafin is intended to suppress post-operative inflammation and reduce the frequency and intensity of the accompanying post-operative complications (Figure 1).

Neutrophil activation and elastase release cause myocardial ischaemia/reperfusion injury in coronary artery bypass surgery. Furthermore, blood contact with foreign surfaces of the extracorporeal circulation activates circulating neutrophils, triggering elastase release and a systemic inflammatory response with pulmonary and renal complications [46]. Peri-operative elafin treatment is expected to attenuate myocardial reperfusion injury and the systemic inflammatory response, leading to improved post-operative cardiac function and alleviation of complications involving other organs.

CAN (chronic allograft nephropathy) occurs at least 3 months after transplantation surgery and is the most common cause of renal graft failure. CAN may be triggered by post-transplantation ischaemia/reperfusion injury, which results in the recruitment of neutrophils and elastase release [47,48]. Elastase inhibition with elafin is anticipated to suppress post-operative reperfusion injury, leading to an amelioration of chronic renal allograft nephropathy and an increase in the functional life of the graft.

Using yeast-derived recombinant human elafin, favourable results have been obtained in animal toxicity studies and a Phase I clinical trial in healthy subjects, in which elafin was applied intravenously. Currently, a Phase II clinical trial, in which patients undergoing transthoracic oesophagectomy received intravenous elafin on the day of surgery, has been completed and further Phase II clinical trials for the use of elafin in coronary artery bypass surgery and kidney transplantation have been designed and approved by the respective authorities.

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