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Ecallantide

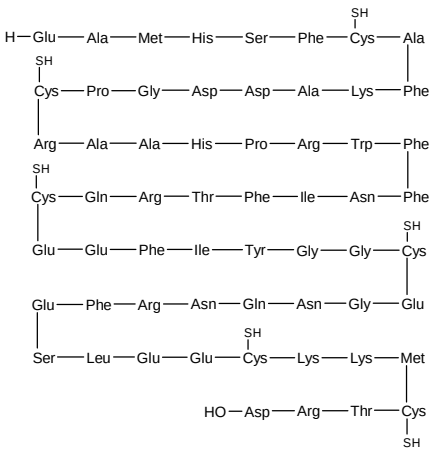
Rec INN; USAN

Plasma Kallikrein Inhibitor
Treatment of Hereditary Angioedema

DX-88
EPI-KAL2

Human plasma kallikrein inhibitor (synthetic protein)

L-Glutamyl-L-alanyl-L-methionyl-L-histidyl-L-seryl-L-phenylalanyl-L-cysteinyl-L-alanyl-L-phenylalanyl-L-lysyl-L-alanyl-L-aspartyl-L-aspartyl-glycyl-L-prolyl-L-cysteinyl-L-arginyl-L-alanyl-L-alanyl-L-histidyl-L-prolyl-L-arginyl-L-tryptophyl-L-phenylalanyl-L-phenylalanyl-L-asparaginyl-L-isoleucyl-L-phenylalanyl-L-threonyl-L-arginyl-L-glutaminyl-L-cysteinyl-L-glutamyl-L-glutamyl-L-phenylalanyl-L-isoleucyl-L-tyrosyl-glycyl-glycyl-L-cysteinyl-L-glutamyl-glycyl-L-asparaginyl-L-glutaminyl-L-asparaginyl-L-arginyl-L-phenylalanyl-L-glutamyl-L-seryl-L-leucyl-L-glutamyl-L-glutamyl-L-cysteinyl-L-lysyl-L-lysyl-L-methionyl-L-cysteinyl-L-threonyl-L-arginyl-L-aspartic acid



$C_{305}H_{448}N_{88}O_{91}S_8$
Mol wt: 7059.8871

CAS: 460738-38-9

EN: 274291

Abstract

C1 esterase inhibitor (C1-INH) activity is reduced in the blood of patients with hereditary angioedema (HAE) and is depleted in patients undergoing cardiopulmonary bypass surgery (CPB). The effect of this insufficiency is activation of the complement system, leading to inflammatory responses, and activation of the kallikrein-kinin system, leading to the swelling and pain associated with acute HAE attacks. Depleted C1-INH and elevated kallikrein are also believed to lie behind the inflammatory injuries and high blood loss associated with CPB surgery. Ecallantide is a recombinant protein inhibitor of kallikrein that is under development for the treatment of acute HAE attacks and to reduce blood loss in patients undergoing CPB surgery.

Background

C1 esterase inhibitor (C1-INH) is a plasma protein of the serpin family that suppresses multiple serine protease-dependent pathways in the blood system, including the complement system, the coagulation cascade and the kallikrein-kinin cascade (also known as the contact cascade). C1-INH inhibits several components of the complement system, including proteases C1r and C1s of the classical complement pathway, thereby regulating the activation of this pathway. C1-INH also inhibits the conversion of prekallikrein to kallikrein in the plasma, which in turn influences the activity of several pathways: kallikrein modulates the activity of the complement system by activation of C5; it releases bradykinin, which in turn stimulates vasodilatation and increased vascular permeability; kallikrein activates factor XII (and C1-INH inhibits it), which contributes to blood clot formation; and kallikrein mediates the release of plasmin, required for the dissolution of blood clots. C1-INH and kallikrein probably do not play a dominant role in the latter two pathways, as individuals with mutations in their *C1INH* gene do not have bleeding abnormalities (1).

Plasma C1-INH activity has importance in the clinical setting because mutations in the corresponding gene cause hereditary angioedema (HAE), a rare autosomal dominant disease (prevalence 1/10,000-50,000). HAE is characterized by acute attacks of edema that occur in any part of the skin or in the mucosa of the respiratory and gastrointestinal tracts. Attacks often last about 3 days. Antifibrinolytics and androgens are used as prophylactics, and where available, human plasma-derived C1-INH is

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used to treat severe acute attacks (C1-INH has not been approved by the FDA). During active attacks, the complement system and plasma kallikrein are activated and bradykinin levels increase. Bradykinin promotes vasodilatation, increases vascular permeability and stimulates various pain receptors, and it is thought to be a major contributor to the inflammation and pain response in angioedema attacks (1-3).

Cardiopulmonary bypass (CPB) surgery is complicated by inflammation-related injury to organs such as the liver and by extensive blood loss. Plasma C1-INH levels are decreased during this type of surgery and the complement system and plasma kallikrein are activated. Kallikrein is further activated by contact with negatively charged surfaces such as the membrane of the oxygen accumulator used in the surgical procedure; hence, its alternative name, the 'contact' cascade. Together, elevated kallikrein and depressed C1-INH are thought to contribute to the inflammatory damage during this type of surgery. Plasma kallikrein also activates fibrinolysis, leading to reduced blood viscosity and potentially to the high blood loss during surgery (4). Aprotinin is an antifibrinolytic agent currently used to reduce blood loss in this type of surgery, but it has little efficacy in reducing the associated inflammatory damage (5). Thus, inhibition of plasma kallikrein is a potential therapeutic strategy for treating conditions associated with C1-INH deficiency, such as HAE and CPB surgery.

Ecallantide (DX-88) is a recombinant peptide inhibitor of kallikrein undergoing phase III clinical trials for the treatment of severe acute HAE attacks and phase II trials for prophylactic use to reduce perioperative blood loss and the need for blood transfusions in patients undergoing CPB surgery. Ecallantide has orphan drug status in the U.S. and the E.U., as well as FDA fast track designation, for the treatment of acute attacks of HAE.

Preclinical Pharmacology

Ecallantide was identified from a phage display library of variants of the first Kunitz domain of tissue factor pathway inhibitor (TFPI), a naturally occurring protease inhibitor that prevents coagulation. The peptide is comprised of 60 amino acids, has a molecular weight of 7054 D and differs from the parent protein by just 7 amino acids. Its K_i for human plasma kallikrein is 25-44 pM and it exhibits little activity against other serine proteases. Ecallantide is produced as a recombinant protein in the yeast *Pichia pastoris* (6-8).

Studies in cultured human umbilical vein endothelial cells (HUVEC) showed that ecallantide binds to the surface of the cells and the cell-bound material retains the ability to inhibit kallikrein (9).

C1INH$^{-/-}$ mice do not have HAE symptoms but do have increased vascular permeability. Ecallantide reversed the increased vascular permeability defect observed in these animals (linear dose-response in the range 10 ng-1 μg i.v.), as did Hoe-140, a bradykinin B_2 receptor antagonist, and human C1-INH (10-12).

The soluble terminal complement complex, also known as SC5b-9, is a component of the complement system that does not cause lysis of target cells but increases the permeability of endothelial cells *in vitro* and of vasculature *in vivo*. The permeabilizing effect of SC5b-9 on HUVEC was partially reversed by ecallantide, Hoe-140 or CV-3988 (a platelet-activating factor [PAF] receptor antagonist), and completely reversed by all three agents in combination (13).

Ecallantide delayed thrombin generation and fibrin formation in whole blood samples from healthy volunteers when the trigger was a contact activator such as celite, kaolin or actin FS, but not when the trigger was tissue factor. This indicated that ecallantide delayed activation of the intrinsic contact-dependent coagulation pathway, but not the extrinsic tissue factor-dependent pathway (14).

Ecallantide caused immediate concentration-dependent (2-14.8 μg/ml) prolongation of the activated partial thromboplastin time (aPTT) in plasma samples obtained from healthy subjects. However, ecallantide had no effect on measures of the tissue factor-dependent pathway, such as prothrombin time (PT), thrombin time and factor VII and fibrinogen activity. Ecallantide concentration-dependently inhibited the amidolytic activity of kallikrein in plasma and in buffer, but not of the serine proteases factor XIIa or factor XIa. Plasmin formation was inhibited by ecallantide (EC_{50} = 5.0 μg/ml), being slightly less active than the hemostatic agent aprotinin (EC_{50} = 1.8 μg/ml). Unlike aprotinin, ecallantide had little effect on fibrinolysis, as determined by euglobulin clot lysis times or fibrin plate assays (15, 16).

The effect of ecallantide on coagulation was determined using blood samples taken from patients undergoing CPB surgery. Addition of ecallantide (5-20 μg/ml) to the samples concentration-dependently extended the activated thromboelastograph (a measure of whole-blood coagulation) reaction time from around 5 min (no drug) to around 12 min (20 μg/ml). The effect was somewhat less pronounced in samples taken presurgery, perhaps reflecting the depletion of contact factors prekallikrein and factor XII as a result of surgery. Another group of patients received the hemostatic agent aprotinin during the operation. The addition of 20 μg/ml ecallantide to the aprotinin-containing blood samples obtained during surgery enhanced the effect of aprotinin, extending the reaction time from 6.2 min to 21 min (17, 18).

In a middle cerebral artery occlusion (MCAO) model of ischemia/reperfusion brain injury in mice, ecallantide protected against reperfusion-related damage. In a transient focal ischemia model, ecallantide (30 μg i.v.) administered at the beginning of the ischemic period reduced the general neurological deficit score (9 *vs.* 11.5 for saline-treated animals), the focal deficit score (11.6 *vs.* 19), brain swelling (3% *vs.* 9%), ischemic volume (6 mm^3 *vs.* 12 mm^3) and the loss of neurons at 24 h. The protective effect was maintained at 7 days. Administration of ecallantide at the beginning of the reperfusion period was also protective, but there was no protection when it was administered 30 min into the

reperfusion period, or in mice with permanent occlusion (no reperfusion) (19, 20).

The efficacy of ecallantide in protecting the lungs from inflammatory injury during CPB surgery was tested in 16 neonatal piglets. All animals underwent 60 min of normothermic surgery with 15 min of cardioplegic arrest, and were treated with ecallantide (n=10; 0.2 mg/kg by bolus at induction and in CPB prime and 0.05 mg/kg/h by continuous infusion) or saline (n=6). Ecallantide improved pulmonary gas exchange, as shown by a reduction in the partial pressure of oxygen in the artery and in the alveolar-arterial oxygen gradient. Other measures of lung function were not affected by ecallantide. This study demonstrated the potential utility for ecallantide as an adjunct for CPB in the neonate (21).

Safety

The safety of ecallantide was assessed following repeated s.c. administration (0.2, 10 and 25 mg/kg every second day for 90 days) to cynomolgus monkeys. Ecallantide prolonged the aPTT at doses of 10 and 25 mg/kg. Toxicity was limited to mild injection-site inflammation. High IgG antibody titers (> 1:26,000) were detected in some of the monkeys following repeated administration at the two higher doses (at least 15-fold higher than doses associated with clinical efficacy). High antibody titers correlated with reduced plasma clearance and half-life of ecallantide, but not with prolongation of aPTT or with other toxicological findings (22).

Combined phase I and II safety results have been reported from 126 individuals (healthy subjects and patients with HAE or undergoing CPB) who had received a total of 165 infusions of ecallantide at various doses. Two subjects reported serious allergic adverse events. One patient with an allergic reaction on the first exposure to ecallantide was tested by immunoblotting techniques and was found to have IgE, IgG, IgM and IgA antibodies to ecallantide. However, a validated ELISA-based assay for IgG and IgE antibodies did not detect anti-ecallantide antibodies in this or other patients who had mild allergic reactions to the drug (23).

Clinical Studies

In a phase I trial, ecallantide (10, 20, 40 or 80 mg by bolus infusion) was administered to 12 healthy subjects. The plasma C_{max} and AUC increased in a dose-dependent manner (C_{max} = 2-15 μg/ml), t_{max} was 10 min and the half-life approximately 90 min. There were no serious adverse events. An *ex vivo* analysis of plasma 1 h after infusion showed prolonged aPTT in samples from 6 of 8 subjects at the 40- and 80-mg doses (7, 15, 16).

Intravenous ecallantide has been studied in three phase II trials in patients with HAE: the EDEMA0 study was an open-label trial in 9 patients with HAE; EDEMA1 was a placebo-controlled, randomized, double-blind, single-escalating-dose protocol in 48 HAE patients; and EDEMA2 was an open-label extension protocol examining the safety and efficacy of multiple treatments with ecallantide in patients with HAE (8, 24).

In the EDEMA0 study, 9 HAE patients received ascending doses of ecallantide (10, 40 and 80 mg by short i.v. infusion). The time to the start of patient-reported symptom relief ranged from 25 min to 3 h and symptoms completely resolved within 2-72 h. Three patients relapsed again 7-15 h after initial regression. One patient had an anaphylactoid reaction, which resolved rapidly upon treatment with adrenaline and an antihistamine. Safety and tolerability were good in the remaining patients (1, 8, 25, 26).

In EDEMA1, 48 patients experiencing acute HAE episodes were randomized to ecallantide (5, 10, 20 or 40 mg/m^2 by 10-min i.v. infusion) or placebo. The primary endpoint, the proportion of patients reporting improvement in symptoms within 4 h of treatment, was significantly greater for the ecallantide group (29 of 40, or 72.5%) compared to the placebo group (2 of 8, or 25%). Ecallantide treatment was associated with a trend for shorter median times to symptom improvement. Most adverse events were mild to moderate and the incidence was similar in the ecallantide (78%) and placebo (87.5%) treatment groups, with no dose-effect. Four patients in the ecallantide group experienced severe adverse events, 2 with rhinitis and breathing problems during the ecallantide infusion period that resolved with antihistamine and other antiallergy medications, 1 with an angioedema attack 21 days after treatment and 1 with abnormally high thrombin times, which resolved on follow-up visits. All 4 tolerated the infusions and completed the infusion period, and none had abnormal bleeding events. Four patients in the ecallantide treatment groups had prolonged aPTT, which normalized within 4 h of the initiation of treatment. None of the 49 patients had anti-ecallantide antibodies as determined by ELISA (1, 8, 27).

In the EDEMA2 study, patients were eligible to receive up to 2 treatments per attack for up to 20 attacks. In an interim report, 60 patients had received ecallantide (5, 10 or 20 mg/m^2 by 10-min i.v. infusion or 30 mg s.c.) for a total of 157 angioedema attacks. Response rates within 4 h of treatment were 90-100%, with comparable results for both routes of administration. The rate of rebound attacks over the period 4-24 h after treatment was 3% in the 10 and 20 mg/m^2 cohorts and 25% in the 5 mg/m^2 cohort. No drug-related serious adverse events were reported (1, 8, 28, 29).

EDEMA3 (DX-88/14) was a multicenter, randomized, double blind, placebo-controlled phase III study of s.c. ecallantide (30 mg) in 72 HAE patients experiencing acute attacks, followed by an open-label phase in which patients received ecallantide (30 mg s.c.) during subsequent acute attacks of edema. The primary endpoint, symptom improvement at 4 h measured by a treatment outcome score developed for HAE attacks, was met (p = 0.021). Secondary outcomes of symptom burden and time to significant overall improvement were also met (median time to improvement of 2.5 h *vs.* 4 h for placebo). In an initial report of the open-label phase of

this study, 19 patients suffered 38 angioedema attacks. Patient-reported improvement of symptoms was achieved in 89.5% of the attacks, and the median time to significant improvement was 56 min. Seventeen adverse events were reported for 11 patients in both phases of the study; 3 mild events (headache, injection-site reaction and injection-site erythema) were considered drug-related (30-32).

EDEMA4 (DX-88/20) is a randomized, double-blind, placebo-controlled phase III safety/efficacy study of ecallantide (30 mg s.c.) that is intended to validate the patient-reported outcome measure used in the EDEMA3 trial (24, 33).

Study DX-88/19 is a nonrandomized, open-label safety/efficacy study of the repeated use of ecallantide (30 mg s.c.) for the treatment of acute attacks of HAE. The study is designed to allow patients continued access to ecallantide, including those completing the EDEMA4 trial, and is intended to support the marketing authorization of ecallantide (34).

Several case reports on the use of ecallantide in HAE have been published. In one case study, a woman who experienced anaphylaxis in response to human C1-INH treatment for acute HAE was successfully treated with ecallantide (50 mg i.v.). The time to significant improvement of symptoms was 30 min, there was no relapse of symptoms in the subsequent 24 h and she had no adverse reaction to ecallantide (35). In another case study, ecallantide (80 mg i.v.) rapidly resolved abdominal pain, nausea and bowel obstruction in a patient with HAE (36). The first patient treated in a multicenter European trial of ecallantide (10, 20, 40 and 80 mg by i.v. infusion) experienced an abdominal HAE attack which was successfully treated with ecallantide (10 mg) (37). A group of doctors at Georgetown University Hospital treated a family with HAE as part of the EDEMA1 trial. Three patients, a father and his 2 daughters, presented with moderately severe acute abdominal attacks. One daughter had resolution of nausea, cramping and rash within 1 h and partial resolution of abdominal distension; the father had an abdominal attack with left hand and scrotal edema, rash and erythema marginatum, with significant resolution of attacks within 2 h, complete resolution of rash and abdominal symptoms, and partial resolution of edema. The other daughter did not respond (38).

The efficacy of ecallantide in reducing blood loss during primary CPB grafting or valve repair/replacement is being studied in a randomized, double-blind, placebo-controlled phase II study (39).

Source

Dyax Corp. (US).

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