Complement depletion with humanised cobra venom factor: Efficacy in preclinical models of vascular diseases

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Summary

The complement system is an intrinsic part of the immune system and has important functions in both innate and adaptive immunity. On the other hand, inadvertent or misdirected complement activation is also involved in the pathogenesis of many diseases, contributing solely or significantly to tissue injury and disease development. Multiple approaches to develop pharmacological agents to inhibit complement are currently being pursued. We have developed a conceptually different approach of not inhibiting but depleting complement, based on the complement-depleting activities of cobra venom factor (CVF), a non-toxic cobra venom component with structural and functional homology to complement component C3. We developed a humanised version of CVF by creating human complement component C3 derivatives with complement-depleting activities of CVF (humanised CVF) as a promising therapeutic agent for diseases with complement pathogenesis. Here we review the beneficial therapeutic effect of humanised CVF in several murine models of vascular diseases such as reperfusion injury.

Keywords

Cobra venom factor, humanised cobra venom factor, CVF, complement depletion, reperfusion injury

Introduction

The complement system is an intrinsic part of the immune system and has important functions in both innate and adaptive immunity. On the other hand, inadvertent or misdirected complement activation is also involved in the pathogenesis of many diseases, and in some cases, represents the major pathogenetic mechanism ([11] and references therein). A few of these diseases include: rheumatoid arthritis (2), lupus erythematosus (3), myasthenia gravis (4), macular degeneration (5), and paroxysmal nocturnal haemoglobinuria (PNH) (6). Several diseases with complement pathogenesis involve the vascular and coagulation systems, with reperfusion injury being a prominent example (7–10).

Cobra venom factor (CVF) is the complement-activating protein in the venom of the Indian cobra (Naja naja) and other cobra species (1, 11). CVF is a structural and functional analogue of complement component C3 (1, 12, 13). CVF activates complement analogously to C3b, the activated form of C3 (14, 15). Both CVF and C3b bind to complement component Factor B. Once in complex with CVF or C3b, Factor B is cleaved by Factor D, resulting in the formation of the bimolecular complex CVF:Bb or C3b:Bb, respectively (1, 16–18). Both complexes are enzymes, called C3/C5 convertase (EC 3.4.21.47), activating both C3 and C5 (15, 19, 20). Whereas C3b-dependent complement activation occurs on the surface of a target cell, and all regulatory mechanisms of the complement system restrict activation to the target site, CVF-dependent complement activation occurs in the fluid phase, and both CVF and CVF:Bb are completely resistant to inactivation (21, 22). Accordingly, CVF will continuously activate C3 and C5, leading to the depletion of serum complement activity.

Ever since it was shown, more than 40 years ago, that CVF can be safely administered to laboratory animals, leading to in vivo complement depletion (23–25), CVF has been used as a tool to study both the biological functions of complement as well as its role in the pathogenesis of diseases by comparing normal (complement-sufficient) animals with complement-depleted animals (1, 11). This approach has led to the delineation of multiple physiological activities of complement as well as the identification of the complement system in the pathogeneses of many diseases.

Given the involvement of complement in the pathogenesis of many diseases, for the last two decades many investigators have devised experimental approaches to inhibit complement with the aim to develop pharmacological agents for therapy of complement-mediated diseases (26–29). Whereas a detailed description of complement inhibitory therapeutics is beyond the scope of this article, complement inhibitors are either directed to prevent the activation of a given complement component or to inhibit the action of an activated complement component. In contrast, we have developed a conceptually different approach to treat complement-mediated diseases. Our approach is not based on inhibition of complement but on depletion of complement, mimicking the complement-depleting activity of CVF (1, 30–32). Whereas CVF...
itself represents a potential therapeutic agent for complement-depletion, and has served as the gold standard in experimental models of disease for evaluating the efficacy of complement inhibitors (33), both the limited availability of its natural source, cobra venom, and its immunogenicity severely limit its clinical usefulness.

To overcome these two limitations, we expressed active recombinant CVF in insect cells (34, 35). We also identified that the C-terminal region of the CVF β-chain, corresponding to the C-terminal region of the C3 α-chain, harbours the crucial structures in CVF allowing its uncontrolled activation of the complement system (36, 37). In a third step, we generated human C3 derivatives with functional features of CVF by replacing the C-terminal portion of the C3 α-chain with the homologous sequence from the CVF β-chain (31, 32, 38, 39). These human C3 derivatives are collectively referred to as “humanised CVF” (hCVF) and represent experimental therapeutic agents for complement depletion in diseases with complement pathogenesis. hCVF protein HC3–1496 is human C3 in which the C-terminal 168 amino acid residues in its α-chain have been replaced with the corresponding CVF sequence (1, 32) (Figure 1). Even within this stretch of 168 amino acids, human C3 and CVF sequences are 44% identical (64% similar); and the overall sequence identity between HC3–1496 and human C3 is 94% (96% similarity) (13, 40). We have shown the efficacy of HC3–1496 as an experimental therapeutic agent in multiple preclinical models of disease including PNH (41), collagen-induced arthritis (30), monoclonal antibody therapy of lymphoma (42), and macular degeneration (43). Here we will review and report on the efficacy of complement-depletion with HC3–1496 in two different preclinical models of reperfusion injury as well as ventilator-induced lung damage.

Myocardial ischaemia/reperfusion injury

To assess the therapeutic potential of complement depletion with hCVF, we used a murine model of experimental myocardial ischaemia/reperfusion injury (MI/RI) (44, 45). Mice were intubated, ventilated, and anesthesia was maintained with isoflurane. The chest was opened and a suture was placed on the left anterior descending coronary artery and tightened. After 30 minutes (min) of ischaemia, the ligation was loosened and the myocardium was reperfused for 4 hours (h). Decomplementation with hCVF protein HC3–1496 at 250 μg/kg was performed by i.p. injection 2 h prior to the induction of anesthesia (46).

Complement-depletion with hCVF protein HC3–1496 was an effective therapeutic approach to reduce reperfusion injury as demonstrated functionally (better ejection fraction), morphologically (smaller infarct size), and immunohistochemically (less deposition of C3b) (Figure 2) (46). Better left ventricular function in animals depleted with hCVF was also demonstrated by an improved fractional shortening as measured by echocardiography (46).

Gastrointestinal ischaemia/reperfusion injury

We used a murine model of gastrointestinal ischaemia/reperfusion injury (GI/IR) to assess the effect of complement-depletion. In this model, mice were anesthetised with isoflurane, and intestinal ischaemia was produced for 20 min by occlusion of the superior mesenteric artery (47–49). Subsequently, 3 h of reperfusion was achieved by removal of the surgical clips. The intestinal permeability was assessed by luminal enteral administration of FITC-conjugated dextran with subsequent determination of FITC-dextran concentrations in serum by fluorescence spectrophotometry (47). Decomplementation with hCVF protein HC3–1496 or natural CVF at 250 μg/kg was performed by i.p. injection two hours prior to the induction of anesthesia. Injection of phosphate-buffered saline (PBS) served as control.

Figure 3 demonstrates that complement-depletion with hCVF protein HC3–1496 results in a significantly decreased serum concentration of FITC-dextran, indicating significantly reduced reperfusion injury compared to PBS-treated animals. Complement-depletion with natural CVF, which served as positive control, resulted in an identical reduction of reperfusion injury (Figure 3).

**Figure 1:** Schematic representation of the chain structures of human pro-C3, pro-CVF, and humanised CVF protein HC3–1496. Human pro-C3 is a single-chain protein of 1641 amino acid residues. It is processed by the removal of four arginine residues into the mature C3 protein consisting of the α- and β-chain. Pro-CVF is a single-chain protein of 1620 amino acid residues. It is processed in the venom gland into the mature three-chain protein involving the removal of four arginine residues, the C3a domain, and the C3dg-like domain. Humanised CVF protein HC3–1496, like human C3, has 1641 amino acid residues. Recombinant production of hCVF in Drosophila S2 cells results in a mixture of a C3-like and a C3b-like two-chain protein (1, 31, 34). The C-terminal 146 amino acid residues of both C3 and CVF constitute the C345C domain which has been shown by x-ray crystallography to bind Bb in the convertase (16, 18). Not surprisingly, this region in CVF harbors the important structures responsible for forming a stable convertase.
Ventilator-induced lung injury

Ventilator-induced lung injury (VILI) is a major course of morbidity and mortality in critically ill patients (50). A murine model of VILI was used to assess the effect of complement depletion with hCVF (51). Mice were anaesthetised with isoflurane and, after a mid-line laparotomy, intestinal ischaemia was produced for 20 min by occlusion of the superior mesenteric artery with surgical clips (47-49). Subsequently, 3 h of reperfusion was achieved by removal of the clips. The intestinal permeability was assessed by luminal enteral administration of FITC-conjugated dextran 4000 (47). The animals were gavaged 5 min prior to ischaemia with FITC-dextran at 200 μg/g of body weight. Blood was obtained by cardiac puncture at the time of euthanasia. Blood samples were allowed to clot at 4°C and centrifuged. Serum FITC-dextran concentrations were determined by fluorescence spectrophotometry (47). Decomplementation with hCVF protein HC3–1496 or natural CVF at 250 μg/kg was performed by i.p. injection 2 h prior to the induction of anesthesia. Injection of PBS served as control. Shown is the uptake of FITC-labelled dextran into the blood stream from the intestine. PBS and natural CVF served as negative and positive controls, respectively.
Conclusion

As demonstrated in the preclinical models of disease described here, temporary ischaemia or mechanical injury both cause vascular tissue damage that leads to subsequent activation of complement. In turn, complement activation causes significant tissue damage well above and beyond the initial tissue damage by ischaemia or mechanical injury. The structural changes induced by ischaemia or mechanical injury that lead to activation of complement are not known. In reperfusion injury, both the lectin pathway and the alternative pathway have been shown to be activated whereas the classical pathway is not, although IgM binding to neoptetopes leads to MBL binding and subsequent complement activation (8, 52). Regardless of the mechanism of complement activation, the complement-dependent tissue damage is demonstrated by morphological and, importantly, functional impairment of the affected organs. In all cases, depletion of complement with humanised CVF prevents the complement-mediated tissue damage.

It is noteworthy to add that no adverse side effects of complement depletion with humanised CVF were observed, neither in the three preclinical disease models reported here nor in any other preclinical disease model (1, 30, 42, 43, 45, 51, 53, 54). Moreover, the only known side effect of massive fluid-phase complement activation by natural CVF is a consequence of the released anaphylatoxins C3a and C5a. Both anaphylatoxins are readily inactivated by carboxypeptidase N to C3a-des-Arg and C5a-des-Arg, respectively. However, C5a-des-Arg retains its ability to activate neutrophils, which have been shown to be sequestered in the lungs, causing acute but fleeting inflammatory lung injury (55–57). Fortunately, humanised CVF lacks C5-cleaving activity and does not generate C5a (1, 31). This is consistent with a complete lack of lung injury in cynomolgus monkeys after intra-arterial hCVF injection into the pulmonary artery (1, 58). Another potential complication is immunogenicity of hCVF. However, recombiantly produced hCVF lacks the unusual oligosaccharides of native CVF (59), and exhibits 94% sequence identity with human C3, along with an identical domain structure (1, 16, 30), suggesting a significantly reduced or potentially absent immunogenicity. Indeed, hCVF displayed no functionally relevant immunogenicity compared to CVF in a murine model of haemophilia A (60).

Accordingly, complement depletion with humanised CVF represents a promising therapeutic strategy to prevent tissue damage and functional impairment in reperfusion injury and ventilator-induced lung damage, as well as other diseases with complement pathology (1).

Conflicts of interest

CWV, DCF, and GLS were former recipients of research grants from Incode Biopharmaceutics, Inc. and previously had a financial interest in the company.

References