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Development of Compstatin Derivative-Albumin Binding Peptide Chimeras for Prolonged Plasma Half-Life

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Introduction

Complement is a powerful arm of the innate immune system and a bridge to adaptive immunity, which plays important roles in antimicrobial defense and apoptotic cell clearance [1]. Under normal conditions, complement activation is tightly controlled by various fluid-phase and cell-surface-bound regulatory proteins. However, inappropriate or excessive complement activation can overwhelm this delicate balance and cause host tissue damage as implicated in many pathological conditions including age-related macular degeneration (AMD), rheumatoid arthritis, sepsis, ischemia-reperfusion injuries and graft-rejection during transplantation etc. [2]. In 1996, our laboratory discovered a peptide named Compstatin (H-Ile-c[Cys-Val-Val-Gln-Asp-Trp-Gly-His-His-Arg-Cys]-Thr-NH_2) that selectively binds to human and primate complement component 3 (C3) and its active fragment C3b, and effectively inhibits complement activation [3]. Many years of optimization have led to the development of a largely improved Compstatin analog (Figure 1, **1**, IC_{50}=205 nM), which is 264-fold more potent than the original peptide [4]. Recently, a Compstatin derivative successfully completed a phase I clinical trial under the name POT-4 (Potentia Pharmaceuticals, Inc.) for the treatment of AMD and showed a highly beneficial safety and toxicity profile. Once injected intravitreally, it forms a gel-like deposit in the eye, which slowly releases active peptide over a prolonged period of time. The size of the deposit is depending on the amount of peptide injected [5]. In contrast to these advantageous properties for local injection in the eye, the relatively short in vivo half-life of Compstatin so far limited its potential for diseases requiring systemic administration. Conjugation of drugs to an albumin binding peptide (ABP: Ac-RLIEDICLPRWGCLWEDD-NH_2, $IC_{50,human}$=467 nM) has been described as a promising approach to significantly increase peptide half-life by taking advantage of the transporter/depot function of the highly abundant serum albumin [6]. Therefore, we have designed two chimeric peptides (**2** and **3**), in which ABP was conjugated to a Compstatin derivative (**1**) in either N-terminal or C-terminal position via a mini-PEG linker (Peptides International, Louisville, KY). Advanced peptide synthesis protocols were used since both Compstatin and ABP contain one disulfide bridge each that has to be formed sequentially. Their activities were investigated using ELISA and surface plasmon resonance (SPR).

Results and Discussion

The conjugated linear peptide chimeras were synthesized manually on MBHA Rink amide resin via Fmoc chemistry. DIC and HOBt were used for all coupling reactions. To avoid shuffling of the two distinct disulfide bonds, the two pairs of cysteine were orthogonally protected with Trt and Acm groups, respectively. After the peptides were cleaved from the resin with concurrent Trt group removal, they were subjected to air oxidation to form the first disulfide bond. The resulting products were purified by reverse-phase HPLC and lyophilized. They were then treated with AgOTf in TFA/anisole to remove the Acm protecting group. The second disulfide bond was formed when the deprotected peptides were treated with a 1N HCl/DMSO (1:1)

1 Ac-ICVW(Me)QDWGHHRCT-NH_2

2 Ac-ICVW(Me)QDWGHHRCT-miniPEG-RLIEDICLPRWGCLWEDD-NH_2

3 Ac-RLIEDICLPRWGCLWEDD-miniPEG-ICVW(Me)QDWGHHRCT-NH_2

*Fig. 1. Sequences of peptide **1** and chimeric peptides **2** and **3**.*

Table 1. Results of ELISA and SPR studies of compstatin analogues

Peptide	IC_{50} *(μM, CP)*	IC_{50} *(μM, AP)*	k_{on} $(10^5, M^{-1}s^{-1})$	k_{off} $(10^{-3}, s^{-1})$	K_D *(nM)*
1	0.22	0.20	9.9	11.0	11.0
2	0.83	0.36	4.2	6.5	15.3
3	0.48	0.40	4.9	7.0	14.5

solution [7]. Reverse-phase HPLC purification provided the final products with >95% purity. The inhibitory activities of the purified chimeras were tested using ELISA in human serum and their kinetic profiles were assessed on immobilized C3b using a Biacore 2000 instrument [8]. The results are summarized in Table 1. Both chimeric peptides displayed an only slightly decreased inhibitory potency (2-4 fold) for both the classical pathway (CP) and alternative pathway (AP) of complement activation. According to the SPR analysis, the drop in binding affinity was even lower (1.3-1.4 fold), which can be primarily attributed to slower k_{on} rates because of the increased size of the peptides. Additional ELISA assays (data not shown) show that the chimeric peptides are able to bind both C3 and human serum albumin simultaneously. The collective data indicate that both chimeras are equally potent in serum. Considering the long half-life of human albumin (20 days), the compound is very likely to have significantly increased plasma half-life in human. To determine the plasma half-life of **3** in mice, the peptide was injected into the mouse tail vein. Plasma samples at different time points (up to 24 hour) were collected and analyzed using LC-MS. Our preliminary results indicate that the peptide is still detectable 24 hours after the initial injection. Thus, the chimeric peptides represent promising candidates for the treatment of a broader range of diseases associated with imbalanced complement activation.

In conclusion, chimeras containing a Compstatin derivative and ABP were successfully constructed using a combination of solid phase and solution phase peptide synthesis techniques. ELISA and SPR assays show that their ability to inhibit complement activation is mostly retained, and the position of peptide **1** in the chimeras does not affect their activity. Preliminary in vivo experimenrts in mice suggest that these chimeras feature a largely improved plasma half-life. Further in vivo investigations in primates are planned and data will be reported in due course.

Acknowledgments

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