C3 Targeted Complement Therapy for Chronic Periodontitis – A Scoping Review

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Aim: Chronic Periodontitis (CP) is a complex disease initiated by inflammation caused by dysbiotic bacterial communities in the subgingival environment. The Porphyromonas gingivalis, a keystone pathogen at low colonization, causes immune subversion of complement component C5aR, leading to complement C3-dependent destructive inflammation responsible for the inflammatory bone loss in CP. Animal studies have shown that targeting complement C3 with its inhibitor like AMY-101 may help reduce inflammatory bone loss in CP. This scoping review elaborates on the role of complement C3 targeted therapy for CP.

Materials and Methods: About 66 original studies were obtained during an initial electronic search in Medline (Pubmed), Scopus, Web of Science, and Embase. About four articles were included in the review after screening the duplicates and reading the full text. Their aims and objectives, drug dosage, route of administration, results, and conclusions were recorded.

Results: Of the four-original research, 3 were animal studies and one randomized Phase IIa clinical trial. They showed that C3 targeted complement therapy reduced the inflammatory and clinical periodontal parameters in CP.

Conclusion: C3 targeted complement therapy may be regarded as a valuable adjunct to non-surgical periodontal treatment for CP. However, the results are still under investigation and require further verification through clinical trials.

Keywords: AMY-101, chronic periodontitis, complement, Cp40, targeted therapy

LIST OF ABBREVIATIONS: Chronic Periodontitis: CP, Porphyromonas gingivalis: P gingivalis, Toll like receptor: TLRs, Non-Human primates: NHPs, Probing Pocket Depth: PPD, Gingival index: GI, Plaque index: PI, Clinical attachment level: CAL, Gingival crevicular fluid: GCF, Interleukin: IL, Receptor activator of Nuclear factor Kappa-B Ligand: RANKL, Osteoprotegerin: OPG, Tartrate Resistant Acid Phosphatase: TRAP, Matrix metalloproteinases: MMP

INTRODUCTION

Chronic Periodontitis (CP) is a complex disease initiated by dysbiotic bacterial communities in the subgingival environment.[1] They disrupt the host response resulting in uncontrolled inflammation, which, together with various genetic, systemic, and environmental risk factors, destroys the supporting structures resulting in tooth loss.[1]

Various metagenomic studies have associated more heterogeneous and diverse microbiota with CP than traditional red-complex bacteria such as Porphyromonas gingivalis (P gingivalis), Treponema denticola, and Tannerella forsythia.[2] Isolation of virulence factors of certain unrelated species in the microbiome of periodontitis subjects indicates a polymicrobial synergy and dysbiosis in the pathogenesis of CP.

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of CP. The *P. gingivalis*, a keystone pathogen at low colonization levels, stimulates the conversion of a symbiotic community to a dysbiotic one associated with inflammation and periodontal bone loss. Lately, *P. gingivalis-associated* immune subversion was related to microbial dysbiosis, followed by complement C3-dependent destructive inflammation responsible for the inflammatory bone loss in CP. Further, *P. gingivalis* impairs the microbial killing capacity of neutrophils and macrophages through crosstalk between the toll-like receptors (TLRs) and C5aR. However, their ability to induce inflammation is not affected. Consequently, the *P. gingivalis* and other bacteria escape the phagocytosis and undergo uncontrolled growth due to the indiscriminate production of inflammatory mediators due to C3 activation.

Animal studies have shown that C3 or C3aR deficient mice resist gingival inflammation and bone loss. Accordingly, therapeutic peptides have evolved to target complement C3 and are being investigated in managing CP. These include very selective and effective C3 inhibitor, Compstatin, Cp40/AMY-101(Amyndas Pharmaceuticals), a small-sized peptidic primate/human-specific C3 inhibitor with favorable pharmacological properties and with ability to penetrate the tissues. The AMY-101 is in Phase IIa clinical trials and has shown notable human safety and tolerability. With this background, the current review details the evidence related to C3 targeted complement therapy for CP.

**Materials and Methods**

The research publications related to the C3 targeted complement therapy for CP were identified in databases like Scopus, Medline (PubMed), Embase, and Web of Science as per the Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping reviews (PRISMA-ScR). A combination of keywords like “Complement C3” AND “inhibitor” OR “Cp40” OR “AMY-101” AND “Periodontitis” was applied during the initial search, resulting in 66 articles. Screening the duplicates led to 32 articles wherein the titles and abstracts were read. It resulted in 8 articles, of which four were excluded after reading the full text.

**Inclusion and exclusion criteria**

The inclusion criteria were full texts of original studies in English related to the application of C3 targeted complement therapy for CP. Any recommendations,
conference proceedings, expert statements, technical reports, reviews, case reports, and non-original papers were excluded.

**DATA EXTRACTION**

The type of study, its aims, objectives, drug dosage, route of administration, results, and conclusions were recorded.

**RESULTS**

1. **Type of studies:**
   Among the four studies included in this review, three were animal studies in non-human primates (NHPs)\(^8\)–\(^\text{10}\) and one randomized, double-blind, placebo-controlled, split-mouth phase IIa clinical trial in humans\(^\text{11}\) [Table 1].

2. **Type of animal model:**
   All the animal studies were done in adult systemically healthy cynomolgus monkeys (Macaca fascicularis) with established periodontitis.\(^8\)–\(^\text{10}\)

3. **Type of C3 complement inhibitor tested:**
   The complement C3 inhibitor (Compstatin) Cp40\(^8\),\(^\text{10}\) or AMY-101\(^9\),\(^\text{11}\) were evaluated in the studies.

4. **The site, dosage, and route of administration:**
   A local intragingival injection of AMY-101 or Cp40 with a 0.1 mg/site dose was administered in NHPs with CP.\(^8\),\(^\text{10}\) The administered frequencies included once or thrice per week for three weeks\(^8\),\(^\text{10}\) or once every 2 or 3 weeks.\(^\text{9}\) In all the studies, therapy was stopped at the sixth week, followed by an observation period of six weeks.\(^8\),\(^\text{10}\) However, irrespective of the frequency of administration, clinical periodontal indices were significantly reduced.\(^\text{9}\) Systemically, AMY-101 injected subcutaneously at a concentration of 4 mg/kg body weight, once 24 hourly for 28 days, significantly reduced the probing pocket depth (PPD)\(^9\).

   In the randomized controlled Phase, IIa clinical trial in humans, about 12 individuals were randomly assigned to escalating dosages of 0.025 mg, 0.5 mg, or 0.1 mg per interdental papilla to determine a safe and effective dose in humans.\(^\text{11}\) Based on the primary outcomes' safety and preliminary efficacy analysis, a 0.1 mg per interdental papilla was further evaluated in the study.\(^\text{11}\)

5. **Influence on clinical periodontal parameters:**
   In NHPs, treatment with Cp40 significantly reduced the gingival index (GI), plaque index (PI),\(^8\) PPD, clinical attachment level (CAL),\(^8\),\(^\text{10}\) and tooth mobility due to bone loss.\(^8\) After the drug withdrawal in the sixth week, improvement continued for a minimum of six weeks.\(^9\),\(^\text{10}\) There was a reduction in gingival inflammation (57–87%), PPD (31–58%), and PI after six weeks of treatment.\(^8\)

   In the human randomized controlled clinical trial, the mean modified gingival index and bleeding on probing were significantly reduced on the twenty-eighth day at the sites treated with AMY-101.\(^\text{11}\) The reduction occurred on the twenty-first day and was maintained for ninety days. Further, there was a continuous PPD reduction in the treated group. It was suggested that reduced plaque levels were associated with host modulation following AMY-101 targeted therapy.\(^\text{11}\)

6. **Influence on biomarkers:**
   In NHPs, thrice-weekly treatment with Cp40 significantly reduced pro-inflammatory [e.g., interleukin (IL)-1\^β, IL-6, IL-8, and IL-17] and osteoclastogenic cytokines [e.g., receptor activator of nuclear factor kappa-B ligand (RANKL)] in the gingival crevicular fluid (GCF) during a six week follow up.\(^8\) The levels of complement components C3a and C5/C5a reduced significantly within one week after treatment. Besides, osteoprotegerin (OPG) (an antagonist of RANKL) was increased in GCF. The cytokine levels were reduced in the sixth week and persisted during the follow-up.\(^8\) The immunofluorescence histochemical evaluation of the biopsied connective tissue adjacent to the alveolar bone revealed the anti-inflammatory effect of Cp40.\(^8\)

**Table 1: Studies evaluating C3 targeted complement inhibition therapy for chronic periodontitis**

| Author            | Type of study | Aims and Objectives                                                                 |
|-------------------|---------------|-------------------------------------------------------------------------------------|
| Maekawa T et al 2016\(^\text{8}\) | Animal study | • Determined the inhibitory role of Cp40* (Intra-gingival injection) on Chronic Periodontitis in non-human primates |
| Kajikawa T et al 2017\(^\text{9}\) | Animal Study | • Evaluated the safety and efficacy of locally increasing the AMY-101**dose and reducing the frequency of administration |
| Bostanci N et al, 2018\(^\text{10}\) | Animal study | • Investigated changes in the composition of inflammatory markers in gingival crevicular fluid before and after local administration of C3 complement inhibitor in non-human primates |
| Hasturk H et al 2021\(^\text{11}\) | Human Study Randomized, placebo-controlled, double-blind, split-mouth phase IIa trial | • Evaluated the efficacy and safety of locally administered complement C3–targeted therapeutic, AMY-101 in adults with periodontal inflammation |

*“* and **“” Third-generation Compstatin analogue and complement C3 inhibitors
At the transcriptional level, it significantly inhibited the expression of gingival IL-1β, IL-6, IL-8, IL-17, and RANKL mRNAs, while the OPG mRNA expression was increased. Tartrate Resistant Acid Phosphatase (TRAP)-positive osteoclasts were also reduced at the treated sites. Subsequently, the RANKL to OPG ratio decreased, which prevented bone loss in CP. Similar results were observed with systemic administration of AMY-101 in NHPs.

Another study in NHPs quantified about 573 proteins with more than two peptides. Both once or thrice weekly administration of Cp40 significantly down-regulated proteins like Factor B and properdin, while three proteins, tetractin, mannan-binding lectin, and vitronectin, were upregulated during the six-week course. Drug withdrawal at six weeks increased levels of more than 50% of the down-regulated proteins. The proteomic fingerprint analysis of local tissue exudate revealed that the alternative complement activation pathway was significantly impacted by C3 inhibition as most related proteins were decreased at six weeks. Besides, leukocyte degranulation was affected.

In the human Phase IIa clinical trial of targeted AMY-101, pro-inflammatory matrix metalloproteinases (MMP)-8 and -9 were significantly reduced in the GCF.

7. Adverse effects:
In NHPs, the therapeutic dose of 0.1 mg/site did not produce gingival irritation. However, a dose escalation to 0.5, 2.5, 5, and 10 mg/site showed that doses equal to or higher than 0.5 mg per site caused mild to moderate inflammation. These reactions were commonly associated with 5 and 10 mg/site doses and caused poorer resolution of clinical periodontal parameters like PPD, CAL, and GI.

In humans, no deaths were reported during the phase IIa clinical trial of AMY-101. Only mild to moderate adverse events unrelated to the drug were reported, like systolic blood pressure increase, arthralgia, fatigue, vomiting, thermal burn, and tooth hypersensitivity. Some adverse events related to drug administration were lymph node pain, gingival erythema, and edema at the injection site, which subsided without intervention.

DISCUSSION
It is evident from the results of the studies included in the review that targeted AMY-101 or Cp40 may be a potent anti-inflammatory therapy for CP. The AMY-101 is a cyclic C3-inhibitory peptide based on third-generation Compstatin analogue Cp40. It produces uninterrupted C3 inhibition and has superior binding affinity, greater inhibitory potency, and plasma half-life in humans than most peptidic drugs. It is suitable for targeted drug delivery due to its ability for initial fast clearance of free peptides followed by slow clearance of C3 bound peptides. It binds tightly to C3, whose levels are increased locally in the gingival tissues leading to delayed clearance and sustained protective effects that last for six weeks after the drug withdrawal at the sixth week.

The AMY-101 binds C3 and blocks its cleavage into C3a and C3b by C3 convertase. Subsequently, it inhibits the amplification of the complement cascade regardless of the triggering pathway, i.e., classical, lectin or alternative. As complement activation products C3a and C5a mediate crosstalk signalling with adaptive and immune cells, C3 is a perfect target for curative modulation of complement and inflammatory pathways involved in CP. Furthermore, the anti-inflammatory action of AMY-101 tips the balance toward host-microbe homeostasis as inflammation and dysbiosis are interdependent and could promote active periodontitis.

Studies in NHPs showed that locally administered Cp40 reduced complement components C3a and C5a, pro-inflammatory and osteoclastogenic cytokine levels, and improved clinical periodontal parameters. Cp40-inhibited gingival NLRP3 mRNA expression in NHPs and reduced IL-1β as NLRP3 inflammasome processes IL-1β. Decreased IL-17 levels lead to reduced RANKL. Cp40 affected local complement levels as reduced C3d and C5a levels were evident in the biopsy specimens. AMY-101 or Cp40 decreased pro-inflammatory and osteoclastogenic cytokines like IL-1 β, IL-17, TNF-α, and RANKL and increased OPG levels in the GCF with reduced osteoclasts, and there was a reversal of RANKL to OPG ratio leading to reduced alveolar bone loss.

Furthermore, proteomic analysis of GCF following Cp40 treatment upregulated the levels of proteins like tetractin, mannan-binding lectin, and vitronectin. The mannan-binding lectin and vitronectin are linked to the complement pathway and its regulation, while tetractin is a plasminogen-binding protein significant for early bone remineralization as a bone matrix protein. Vitronectin is significant for cell migration, adhesion, tissue repair, and downregulation of membrane attack complex. Complement inhibition may restore bone and tissue homeostasis due to significant changes in the levels of proteins involved in tissue repair and bone mineralization. Furthermore, Factor B and properdin, critical regulators of the
Alternative complement pathway were downregulated due to inhibition of C5a as it triggers properdin release from neutrophils. Besides, neutrophil degranulation was inhibited, preventing tissue damage.\[10\]

Owing to these beneficial effects of C3 targeted complement therapy, it may be a valuable adjunct to routine non-surgical periodontal therapy. In medicine, diabetic kidney disease and severe COVID-19 were managed with this therapy.\[12,15,17\] A potential concern regarding this therapy is plausible host antimicrobial defense impairment with long-term complement inhibition.\[9\] The limitations of this review are that the results were preliminary and mainly derived from the studies on NHPs. Only one randomized controlled clinical trial in humans was included in the review.

**Conclusion**

It is evident from the included study results that C3 targeted complement therapy may be a valuable adjunct to the non-surgical management of CP due to its anti-inflammatory and antimicrobial effects. Besides, conditions like diabetes mellitus and rheumatoid arthritis associated with CP may be managed with this therapy. However, these results are still under investigation and require further verification through clinical trials.

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Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**Author’s Contribution**

Not applicable.

**Ethical Policy and Institutional Review Board Statement**

Not applicable.

**Patient Declaration of Consent**

Not applicable.

**Data Availability Statement**

Not applicable.

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