

Pathogenesis and Targeted Biologic Therapies for Thyroid-Associated Orbitopathy: A Review

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Abstract: Purpose: To review the pathogenesis and current targeted biologic therapies of Thyroid-associated orbitopathy (TAO). Methods: Pubmed, Cochrane Library databases, Wanfang database and recent relevant journal articles were searched. Results: TAO, a potentially vision-threatening disease, is the most common extrathyroidal manifestation of Graves' Disease (GD). The complex pathogenesis of TAO involves in multiple factors, which has puzzled scientists for many years. With emerging advances in pathogenesis of TAO, novel therapeutic targets have been discovered. For example, rituximab (that acts against CD20), tocilizumab (that acts against the IL-6 receptor), teprotumumab (that blocks IGF-1R) and TNF-inhibitors have proven to be useful and safe therapeutic options in TAO treatment. Conclusions: Targeted biological agents can provide many benefits for TAO treatments. And there is still a long way to go before the potential molecular pathogenesis and novel treatment options of GO are revealed.

Keywords: Thyroid-Associated Orbitopathy, Pathogenesis, Monoclonal Antibodies

1. Introduction

Thyroid-associated orbitopathy, also referred to as Graves' Ophthalmopathy (GO) or thyroid eye disease (TED), is a disordering autoimmune condition which mainly affects orbit and ocular adnexa^[1]. It is the most frequent extrathyroidal feature of Graves' Disease (GD) and the annual incidence of GO is estimated at 19 cases of 100000 persons^[2]. Aberrant epigenetics, including DNA methylation, non-coding RNAs and histone modification, is considered to be closely associated with the incidence, process, outcomes of GO^[3]. As a consequence of improved research techniques and well-developed diagnostic methods, we have a better understanding of the clinical manifestations of TAO. Usually, clinical assessment of GO is characterized by grading disease activity and severity^[4]. The generally described course of TAO is an active inflammatory phase which can last on 6 months to 3 years, followed by a chronic, stable and fibrotic phase^[5]. The severity of TAO mainly depends on the degree of diplopia, dry eye, optic neuropathy, soft-tissue changes and impact on patient's quality of life^[4].

Although the pathogenesis of GO is not yet completely understood, it still provides many thoughts for the treatment of GO. The current established therapies for TAO include glucocorticoid, immunosuppressive agents, orbital radiation and surgery. Over the last decade, we have found that monoclonal antibodies, such as rituximab, tocilizumab, teprotumumab, can alleviate symptoms and improve outcomes of TAO. Hence, this review mainly focuses on the pathogenesis and targeted biological therapies for TAO.

2. Methods

The authors searched in Pubmed, Cochrane Library databases, Wanfang database for following key words: pathogenesis of TAO or GO, treatment for TED or GO, monoclonal antibody. And English articles published since 1992 were selected. Some articles were added based upon the references of the initial articles.

3. Results

3.1 Pathogenesis of TAO

TAO is the most common extrathyroidal manifestation of thyroid dysfunction and the pathogenesis of TAO involves a series of multifocal factors^[6]. Genetic susceptibility and environmental factors may contribute to the occurrence of autoimmune thyroid disease^[7]. Although the pathophysiology of TAO is not yet completely understood, orbital fibroblasts (OFs) still act as key players in this disease^[8]. In addition, autoantigens, lymphocytes, cytokines and fibrocytes are also associated with the onset of TAO^[9].

Several previous studies have confirmed that thyrotropin receptor (TSH-R) is expressed in orbital fibroblasts of both healthy individuals and patients with GO^[10-12]. And clinical studies demonstrate that the higher prevalence of thyrotropin receptor antibodies (TRAb) has been detected in patients with GO^[13, 14]. Through the binding of TSH-R by TRAb, several signaling pathways of OFs are activated. Among of them, there are two main pathways: phosphoinositide 3-kinase/Akt signaling cascade and adenylyl cyclase/cAMP pathway, which cause the enhancement of both hyaluronic acid synthesis and adipogenesis^[15]. In addition, further evidence has indicated that insulin-like growth factor(IGF)-1 receptor(IGF-1R) is expressed on the surface of OFs as well as T and B cells in patients with GO and the density of IGF-1R is 3 fold higher than their normal counterparts^[16, 17]. And there is a similar prevalence of IGF-1R antibodies in patients with GO and healthy controls^[18]. It has been suggested that IGF-1R plays an important role in defining T and B lymphocytes phenotypes and function, mediating HA synthesis and regulating the aberrant behavior of lymphocytes in GO^[19]. However, the autoantigenic roles of TSH-R and IGF1-R still remain controversial. These two receptors could be considered as “partners” in the pathogenesis of TAO^[19].

Lymphocytes also play significant roles in the pathogenesis of TAO through cellular and humoral immunities. B cells could migrate to the orbit and be activated by recognizing autoantigens on the surface of OFs through B cell receptors (BCR). Meanwhile, the combination of membrane-bound CD40L on T cells and CD40 expressed on B cells also provides the signal for B cell activation^[20]. In patients with early onset autoimmunity thyroid disease, thyroid antigen-reactive B cells express higher levels of the activation marker CD86 than controls^[21]. Activated B cells differentiate into plasma cells that can secrete antigen-specific antibodies^[22]. Besides, B cells act as antigen-presentation cells during the initiation of autoimmune process and produce multiple cytokines including IL-4,IL-6,IL-10,TGF- β ,INF- γ , lymphotoxin- α ^[22]. T cells recognize major histocompatibility complex (MHC) proteins on the surface of antigen-presenting cells through T cell receptors (TCRs),which provide the first signal of T cell activation^[23]. The interaction of CD28 on the T cell surface with B7 molecules on B cells is also important for activating T cells^[24]. Activated T cells, mostly CD4+ helper T cells, produce inflammatory cytokines, express adhesion molecules, and recruit more lymphocytes^[25]. Sensitized T lymphocytes recognize autologous orbital antigens from patients with thyroid eye disease^[26], leading to the proliferation and differentiation of OFs and lymphocytes.

Previous studies have shown that a variety of cytokines form a precise network to coordinately regulate autoimmune responses of TAO. In early phase of this disease, Th1 cells predominate and produce interleukin(IL)-2,interferon(IFN)- γ and tumor necrosis factor(TNF)- α , whereas Th2 cells that can secrete cytokines IL-4,IL-5 and IL-10 are dominant in longer duration of TAO^[27]. These cytokines have different and various effects. For example, IFN- γ upregulates CD40 expression on OFs and fibrocytes; IL-2 improves the ability of proliferation of T cell clones^[28]. In addition, IL-17A producing Th17 cells are newly identified to participate in the inflammatory process of TAO. IL-17A induces the secretion of inflammatory cytokines and promotes the extracellular matrix (ECM) synthesis of OFs^[29].

In our current concept of the disease, OFs are regarded as the key target and effector cells in the pathogenesis of TAO. Under the stimulation of cytokines and autoantibodies, activated OFs produce inflammatory molecules to regulate orbital inflammation^[30]. In the meanwhile, OFs can exhibit enhanced proliferative ability and ECM (particularly HA) production capacity^[30]. It has been illustrated that Thy-1- OFs are capable of adipocyte differentiation^[31], while Thy-1+ subsets differentiate into myofibroblasts^[32]. Both types of OFs can produce ECM and contribute to orbital disease process.

Fibrocytes are derived from bone marrow and also involved in the pathogenesis of GO. They express a series of surface markers, including CD34,CD45,CXCR4 and TSHR.^[33] Further Studies have demonstrated that CD34+ fibrocytes may infiltrate into orbital tissue as well as convert into CD34+ OFs in patients with GO^[33, 34]. Moreover, Tong Wu et al. suggest that TSH and CD40 can induce fibrocytes to secrete IL-12, promoting Th1 cell differentiation and production of Th1 cytokines^[35].

Overall, the pathogenesis of TAO is a complicated process involving multiple factors. And with the development of pathophysiology of GO, therapeutic strategies for TAO based on autoimmune responses is rising.

3.2 Targeted Biologic Treatments for TAO

3.2.1 Rituximab (RTX)

Table 1: RCTs and cohort studies for RTA.

Author	Type of study	Number of patients	Intervention	Follow-up weeks	Outcomes indexes	Efficacy
Savino G et al. ^[42]	RCT	20	Intraorbital RTX in both eyes at a dose of 10mg once a week for a month vs iv methylprednisolone of 500mg for 6 weeks and 250mg for 6 weeks	80	CAS, NOSPECS indexes, proptosis, diplopia, TRAb	Yes
Salvi M et al. ^[43]	RCT	32	Initially 1000 mg RTX, twice at a 2-week interval, a single 500-mg RTX after the amendment vs iv methylprednisolone	24	CAS, NOSPECS class, proptosis, lid fissure, TMS, GO-QOL	Yes
Li J et al. ^[45]	RCT	217	131I treatment vs iv MP (500mg) once a week for 16 weeks, next to 131I treatment vs iv RTX of 1000mg for the first time and 500mg RTX at a two-week interval in combination with 131I treatment	24	Hyperthyroidism treatment outcomes, orbital volumetry, ophthalmic assessments, serum cytokine levels, and adverse effects	Yes
Erdei A et al. ^[46]	Cohort study	5	Iv RTX of 375mg/m ² body surface area weekly for 4 weeks	268	CAS, TRAb	Yes
Mitchell AL et al. ^[47]	Cohort study	9	Iv RTX (1000mg*2 or 500mg*2) at fortnightly intervals	116	CAS, TBII levels, NOSPECS class,	Yes
Insull EA et al. ^[48]	Cohort study	12	Iv RTX (100mg*2) at 2 weeks apart	26.4	CAS, VIS overall severity scores, TRAb	Yes
Du Pasquier-Fediaevsky L et al. ^[49]	Cohort study	15	Iv RTX of 1000mg twice at a 2-week interval	24	CAS, proptosis, lid fissure width, eye motility, TRAb	Yes
Eid L et al. ^[50]	Cohort study	14	Iv RTX of 1000mg twice at a 14-day interval	24	CAS, TES, eye motility, relapse, safety	Yes
Deltour JB et al. ^[51]	Cohort study	32	Iv RTX of 1000mg on day 1 and day 15	24	CAS, diplopia, visual acuity, adverse reactions	Yes
Silkiss RZ et al. ^[52]	Cohort study	12	Iv RTX of 1000mg twice at 2-week apart	52	CAS, TAOS, TSI, TSH	Yes
Salvi M et al. ^[53]	Cohort study	12	Iv 375mg RTX/m ² at 2-week apart for total 4 times	70-75	TRAb, euthyroid patients	Yes
El Fassi D et al. ^[54]	Cohort study	10		100		Yes

RTX is a genetically-engineered monoclonal antibody which contains an antigen-binding region of murine origin and a constant region of human immunoglobulin G1^[36]. It can specifically recognizes the

CD20 antigen, which is widely expressed on B lymphocytes and B cells^[37]. The binding of RTX to CD20 is able to activate cell destruction through these mechanisms of apoptosis, complement activation and cell-mediated cytotoxicity^[38]. After infusion of RTX, B cells could be completely depleted in a matter of days and reach to normal levels in 9-12 months in plenty of diseases^[39]. Although RTX can cause B cell depletion, it appears to affect peripheral-blood immunoglobulin concentrations only minimally^[40].

Recently, a quantity of beneficial effects of RTX in autoimmune diseases (such as RA, SLE, ITP) have been reported, which makes it more common in clinical practice. Marius N Stan et al. performed a prospective, randomized, double-masked, placebo-controlled trial enrolled 25 patients with moderate-to-severe GO, which showed there were no difference between RTX and placebo groups in the primary endpoint (a reduction in CAS by ≥ 2 points at week 24) or any secondary endpoints^[41]. On the contrary, randomized controlled trials (RCTs) conducted by Savino G et al.^[42] and Salvi M et al.^[43] have exhibited that RTX was an efficacious, well-tolerant and safe therapy for GO patients. Recently, a meta-analysis about RTX has demonstrated that intravenous RTX treatment provides acute and long-lasting beneficial effects on reductions of CAS and TRAb, limited influence on proptosis^[44]. And in recent years, several RCTs and cohort studies have been made to prove that RTX treatment is effective in TAO (Table 1)^[42,43,45-54]. Therefore, there is still a long way to go until we determine the optimal dosing and biological mechanism of RTX treatment for TAO.

3.2.2 Tocilizumab (TCZ)

Tocilizumab, a humanized recombinant monoclonal antibody, specifically binds and blocks membrane-bound interleukin-6 receptors^[55]. Several cell types (including T and B lymphocytes, monocytes and fibrocytes) can secrete cytokine IL-6. And it plays an important role in the pathogenesis of GO by increasing expression of autoantigens within orbital tissues^[56]. TCZ can suppress the autoimmune process by affecting IL-6/sIL-6R system^[57].

TCZ has been approved by USFDA for the treatment of patients with rheumatoid arthritis, giant cell arthritis and other autoimmune diseases^[58]. In recent years, several clinical studies have evaluated the effects of TCZ on clinical symptoms and signs of GO. And as the 2021 EUGOGO clinical practice guidelines, TCZ may be given consideration as a second-line treatment for moderate-to-severe and active glucocorticoid-resistant GO^[59].

There is only one double-masked, placebo-controlled, randomized clinical trial which involved 32 adults with moderate-to-severe corticosteroid-resistant GO^[60]. Patients were randomly assigned to receive either TCZ (8mg/kg for intravenous infusion, every 4 weeks for 16 weeks) or placebo. The primary outcome was the proportion of patients with a decrease in CAS by ≥ 2 points from baseline to week 16. The secondary outcomes included the proportion of recipients with a reduction in CAS by at least 2 at week 40, the proportion of recipients showing a CAS less than 3 (at week 16 and 40) as well as the GQ-QOL score. The primary outcome was met by 93.3% of patients receiving TCZ compared with 58.8% receiving placebo. And significant changes can be observed in secondary outcomes. This trial proposed that TCZ offers a meaningful improvement in activity and severity of GO^[60]. In addition, an observational multicenter study demonstrated that TCZ can provide remarkable improvement in CAS, best-corrected visual acuity (BCVA) and intraocular pressure (IOP) for patients with glucocorticoid-resistant GO^[61]. A retrospective longitudinal study conducted by reviewing the medical records at a single center showed that TCZ is beneficial for the treatment of TAO^[62]. Amin Bennedjai et al. documented that those 7 patients (100%) treated with TCZ and 9 patients (64%) receiving RTX achieved the primary outcome (a reduction of at least 2 points of CAS)^[63]. In short, intravenous TCZ is an effective and safety therapeutic option for patients with GO.

In spite of the well-tolerance of intravenous TCZ in GO patients, intravenous infusion is time-consuming and resource-constrained. Prior studies have elucidated that the efficacy and safety of subcutaneous TCZ are almost congruent with intravenous administration in patients with rheumatoid arthritis^[64]. Its off-label subcutaneous use has been described in several GO cases that all exhibited clinical improvement in thyroid eye disease activity^[55, 65, 66]. Therefore, the therapy of TCZ in GO still needs further research.

3.2.3 Teprotumumab (TPT)

Teprotumumab, market as Tepezza, is a fully human immunoglobulin G1 monoclonal antibody that binds to extracellular α -subunit domain of IGF-1R with high affinity and specificity^[67]. Furthermore, it has been shown that TPT attenuates TSH/M22-induced TNF- α production and reduces TSHR and IGF-1R display on fibrocytes^[67, 68]. The pharmacokinetics of TPT can be described as a two-compartment population pharmacokinetic model. Patients with TAO were treated with TPT, 10mg/kg for the first

intravenous infusion and 20mg/kg every 3 weeks thereafter^[69, 70]. The teprotumumab steady-state AUC and mean peak concentration were 138mg·h/ml and 632µg/ml respectively^[69, 70]. The TPT central and peripheral volume of distribution were 3.26L and 4.32L separately. The estimated clearance of TPT was 0.27L/day and the elimination half-life of TPT was 20 days according to the population pharmacokinetic model^[69, 70].

TPT initially was developed to suppress tumor growth. In 21 January 2020, TPT was the first approved drug for treatment of TAO by USFDA based on the results of two studies (Study 1 and 2)^[69, 71]. NCT01868997 is a randomized, multicenter, double-masked, placebo-controlled phase 2 clinical trial to determine the efficacy and safety of TPT^[72]. A total of 88 patients with active, moderate-severe TAO were randomly assigned to receive TPT (10mg/kg for initial dose, followed by 20mg/kg for the remaining seven infusions, administered intravenously once every 3 weeks) or placebo. The primary end point was the response that was defined as a reduction of 2 point or more in the Clinical Activity Score (CAS) and a reduction of 2mm or more in proptosis at week 24. Compared to 20% of placebo recipients, 69% of TPT recipients had a response at week 24 (P<0.001). The time to the first response was obviously shorter in the TPT group than in the controlled group (P<0.001). And at all time points, the Graves' ophthalmopathy-specific quality-of-life (GO-QOL) visual-functioning score increased markedly in TPT group. All in all, a course of TPT treatment provides clinical benefits for patients with active, moderate-severe TAO.

NCT03298867 is a randomized, double-masked, placebo-controlled phase 3 multicenter trial involved in 83 patients with active, moderate-severe GO^[73]. They were randomly assigned in a 1:1 ratio to receive intravenous TPT (10mg/kg for the first infusion and 20mg/kg thereafter) or placebo once every 3 weeks for a total of eight infusions. At week 24, the proptosis response (the primary outcome, defined as a reduction in proptosis of ≥2mm in the study eye without a corresponding increase of ≥2mm in the fellow eye) was 83% in the TPT group vs 10% in the placebo group (P<0.001). All secondary outcomes were significantly better among patients with TPT than with placebo, including overall response (a reduction in proptosis of ≥2mm plus a reduction in CAS of ≥2 points), CAS of 0 or 1, the mean change in proptosis across trial visits, diplopia response (a reduction in diplopia of ≥1 grade) and the mean change in GO-QOL overall score (P<0.001 for all). Moreover, the orbital imaging performed in 6 patients with TPT therapy showed reductions in extraocular muscle volume and/or orbital fat volume. The results of the current trial revealed that TPT treatment were more effective than placebo in regard to proptosis response, diplopia response, quality of life and so on among patients with active, moderate-severe TAO.

However, in the NCT01868997 trial, the incidence of all adverse events (including nausea, muscle spasms, diarrhea, hyperglycemia, paresthesia and hearing loss etc.) was 74% in TPT group compared with 73% in placebo group^[72]. In the NCT03298867 trial, the incidence of all adverse events was 85% in TPT group and in placebo group was 69%^[73]. Therefore, it is necessary to evaluate the long-term results and safety of TED.

3.2.4 Inhibitors of TNF-α

Infliximab, consisting of the antigen binding domain and the constant portion of human IgG1, is a chimeric monoclonal antibody against TNF-α.^[74] Several publications have showed successful use of infliximab in orbital inflammatory disease^[75-78]. And it appears to be a useful drug for TAO.

Adalimumab is a purely human monoclonal antibody which specifically binds to TNF-α and has cytotoxic effects on cells that express TNF-α receptors^[74]. Previous studies manifest that adalimumab may decrease expression of proinflammatory cytokines^[79] and have a role as steroid-sparing agent for patients with TAO^[80].

Etanercept, a fusion protein composed of the extracellular ligand-binding portion of TNF receptor linked to the constant portion of human IgG1, binds to both TNF-α and TNF-β to inhibit the interaction of TNF with their receptors^[74]. D Pavidans et al. and Olivera Boskovic et al. suggest that etanercept may suppress the symptoms and clinical signs of GO^[81, 82].

There is still no sufficient evidence about the relationship between TNF-α inhibitors and TAO. Hence, randomized controlled studies are needed to further evaluate effects for GO of TNF-α inhibitors.

4. Discussion

GO, resulting from a complex interaction of endogenous and exogenous risk factors^[83], can lead to typical ocular symptoms, vision loss and decreased quality of life. The pathogenesis of TAO refers to

many factors including orbital fibroblasts, TSH-R, IGF-1R, lymphocytes, cytokines, fibrocytes. As a result of substantial progress in pathogenesis of GO, several potential therapeutic targets have been discovered. Monoclonal antibodies against CD20, IL-6R, IGF-1R, TNF- α have been developed to contribute to the treatment of GO. Many clinical studies have demonstrated that targeted biological therapies provide significant benefits for GO patients. Up to now, TPT is the only approved drug for treatment of moderate-to severe active GO patients by USFDA. Therefore, it is still necessary to investigate the unknown molecular pathogenesis and long-term effects of targeted biological therapies.

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