

Immunomodulatory Effects of Topical Ophthalmic Tofacitinib (Cp-690,550) in Dry Eye Disease

Jing-Feng Huang, PhD

President, La Jolla BioConsulting LLC, San Diego, California, US

Abstract

Ocular surface inflammation is thought to play a key role in dry eye pathogenesis and clinical manifestation. Multiple inflammatory cytokines signal through intracellular janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. Tofacitinib (CP-690,550), a potent and selective inhibitor of JAKs, has been approved for the treatment of rheumatoid arthritis in the US and is in clinical development for the treatment of other autoimmune diseases. Topical ophthalmic tofacitinib was evaluated and has shown immunomodulatory activity in reducing ocular surface inflammation in dry eye, thus, has the potential to improve ocular surface health in dry eye.

Keywords

Immunomodulatory, inflammation, dry eye, ocular surface, JAK, tofacitinib, CP-690,550, cytokine

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Correspondence: Jing-Feng Huang, PhD, 4653 Carmel Mountain Road, Suite 308-245, San Diego, CA 92130 E: jingfeng@ljbioconsulting.com

Cytokines are not only critical for host defense and immunoregulation, but are also major players in the immunopathogenesis of inflammatory and autoimmune diseases. Janus kinases (JAKs) are critical for the intracellular signaling of a large family of cytokines. Tofacitinib (CP-690,550) is a potent and selective inhibitor of JAKs. Oral tofacitinib has been approved for the treatment of rheumatoid arthritis (RA) in the US and is in clinical development for the treatment of other autoimmune diseases. This review highlights the recent observation that topical ophthalmic tofacitinib has immunomodulatory activity in dry eye, indicating that ophthalmic tofacitinib has the potential to reduce ocular surface inflammation and improve ocular surface health in dry eye disease (DED).

Tofacitinib—A Janus Kinase Inhibitor

JAK is a family of intracellular non-receptor tyrosine kinases that are associated with the cytoplasmic domain of Type I and II cytokine receptors. There are four members in the JAK family: JAK1, JAK2, JAK3, and TYK2.¹

Upon cytokine binding and activation of the cognate receptors on the cell surface, JAKs phosphorylate the cytoplasmic tail of the receptor and allow the recruitment of various signaling molecules, including members of the signal transducer and activator of transcription (STAT) family. STATs, phosphorylated by JAKs, dimerize, translocate to the nucleus, and regulate the expression of numerous genes. Cytokine receptors are paired with different JAKs, for example, interferon gamma (IFN- γ) signals through JAK1/JAK2, interleukin (IL)-2 signals through JAK1/JAK3, IL-12 and IL-23 signal through JAK2/TYK2, while IL-6 signals through JAK1/JAK2/TYK2.

Many of the major cytokines that are critical to immune cell activation and proinflammatory cytokine production use the intracellular JAK/STAT pathway to exert their effects (and multiple inflammatory cytokines signal through pathways involving JAK1 and JAK3) rendering them amenable to therapeutic blockade with JAK inhibitor.²

Tofacitinib (former CP-690,550, tasocitinib), a small molecule selective inhibitor of the JAK family, binds to the active site of JAK and prevents JAK activation. Inactive JAK is unable to phosphorylate STAT, thus tofacitinib inhibits the JAK pathways preventing translocation of STAT and reducing cytokine signaling from inside the cell. Tofacitinib inhibits JAK3 and JAK1 and, to a lesser extent JAK2, exhibits functional selectivity for JAK1/3 and JAK1/2 signaling over JAK2/2 signaling in a cellular setting. It has little effect on TYK2, and has high selectivity and specificity, sparing other protein kinases.^{2,3}

Development of Tofacitinib for the Treatment of Immune Mediated Diseases US Food and Drug Administration Approval for the Treatment of Rheumatoid Arthritis

Oral tofacitinib (Xeljanz[®]) has demonstrated efficacy and a manageable safety profile in the treatment of RA and in the US is indicated for the treatment of adult patients with moderately to severely active RA who have had an inadequate response or intolerance to methotrexate. It may be used as monotherapy or in combination with methotrexate or other non-biologic disease-modifying antirheumatic drugs (DMARDs).^{4,5}

Tofacitinib has shown to reduce serum levels of IL-6 and IL-8 in patients. However, the mechanism of action (MOA) of tofacitinib in RA is not completely clear, but is thought to be through direct suppression of IL-17 and IFN- γ production and CD4+ T cell (presumably Th1 and Th17) proliferation in local inflamed tissue (synovium), resulting in a reduced level of IL-6 and IL-8.^{6,7}

Other Autoimmune and Inflammatory Indications

Tofacitinib is also in clinical development for the treatment of various immune-mediated disorders and transplant rejection. Oral tofacitinib was initially evaluated as an immunosuppressive agent in transplants and showed efficacy in renal transplant patients.⁸ Oral tofacitinib is currently in phase III in psoriasis and psoriatic arthritis.⁹ It has also been evaluated and showed promising efficacy in inflammatory bowel disease (IBD), in particular in ulcerative colitis (UC) patients, and is in on-going trials in UC and Crohn's disease.

Topical tofacitinib has also been tested for the treatment of chronic plaque psoriasis and showed promising efficacy: 2 % tofacitinib ointment was administered twice daily (BID) for 4 weeks in a phase IIa trial.¹⁰

Ocular Surface Inflammation in Dry Eye Disease

DED is a highly prevalent condition, affecting an estimated 25 million people in the US and 370 million patients worldwide.^{11,12} Symptoms of ocular discomfort, irritation, and blurred vision often affect dry eye patients' quality of life. Reading speed is reduced in severe DED patients, and visual functioning related to common tasks of daily activities, such as reading, watching television, driving, and using the computer are affected by DED in patients.¹³⁻¹⁶

Significant progress has been made toward identifying and characterizing the underlying inflammation in the ocular surface in DED through clinical and preclinical research, which has led to better understanding of the major role of inflammation in DED pathogenesis and clinical manifestation. An increasing body of clinical and preclinical evidence implicates specific cytokines and cell subsets as drivers of pathogenesis in DED.¹⁷⁻²⁰

Higher levels of inflammatory mediators and cytokines were detected in tear fluids collected from DED patients, such as IFN- γ , IL-17, IL-12, IL-23, IL-1, IL-6, IL-8, and matrix metalloproteinase 9 (MMP-9). Levels of RNA transcripts of many of them were also found higher in conjunctiva surface cells obtained from DED and Sjogren patients.²¹⁻²⁴

It is thought that desiccating stress provokes ocular surface tissues to secrete inflammatory cytokines, such as IL-1, IL-12, IL-23, IL-6, and TNF- α , which in turn lead to activation and subsequent expansion of pathogenic IFN- γ -secreting T helper (Th)-1 cells and IL-17-secreting Th17 cells.^{19,25-27}

As an important proinflammatory Th1 cytokine, IFN- γ is known to induce cell surface expression of major histocompatibility complex (MHC) II, intercellular adhesion molecule 1 (ICAM-1), and a number of co-stimulatory and adhesion molecules in epithelial cells and other cell types, thus enhancing immune activation. IFN- γ has also been shown to play a pivotal role in promoting apoptosis and squamous metaplasia of the ocular surface epithelia in DED.^{25,27} In an experimental dry eye model, IFN- γ induces corneal epithelial apoptosis through activating the apoptotic pathway. An elevated tear level of IFN- γ has also been associated with squamous

metaplasia of the ocular surface epithelia in dry eye. In the conjunctival epithelium in a dry eye model, IFN- γ decreased conjunctival goblet cell (GC) density and increased expression levels of cornified envelope precursor protein SPRR-2 and other epithelial differentiation-related proteins.

Cell surface expression of human leukocyte antigen-DR (HLA-DR), a MHC class II molecule, is higher in conjunctival epithelial cells obtained from DED patients than from normal eyes.^{28,29} Similarly, ICAM-1 (an adhesion molecule), CD40 and CD49L (co-stimulatory molecules), FAS and FAS-L (apoptosis-inducing molecules), and CCR5 (a chemokine receptor) were also expressed higher in conjunctival epithelial cells from dry eyes patients.³⁰ These cell surface molecules do not express or only express at low levels in healthy epithelial cells and are known to be induced upon activation or by certain inflammatory signals such as IFN- γ . MHC II molecules normally express only on professional antigen presenting cells and they are critical for the interaction with CD4+ T cells. ICAM-1 as an adhesion molecule is a ligand for lymphocyte function-associated antigen-1 (LFA-1) (integrin), which is a receptor found on leukocytes. When activated, leukocytes bind to endothelial cells via ICAM-1/LFA-1 and then transmigrate into tissues. CCR5 is a receptor for chemotactic cytokines: CCL5 (also known as Regulated on Activation, Normal T cell Expressed and Secreted [RANTES]), CCL3, and CCL4 (macrophage inflammatory protein [MIP] 1 α and 1 β). CCL3, 4, 5 are inflammatory chemokines mediating the recruitment of mononuclear cells. Treatment with cyclosporine for 3 and 6 months reduced the expression of HLA-DR and ICAM-1 in dry eye patients.³¹ Thus, HLA-DR has been used as an inflammation marker for the ocular surface in DED.

It is thought that the Th17 cytokine IL-17 promotes corneal epithelial barrier disruption. Interaction of IL-17 with its receptors on the ocular surface cells leads to epithelial damage through increased secretion of inflammatory cytokines and MMPs. Increased levels of MMP-3 and MMP-9 were found in tear fluid from DED patients, and increased MMP-9 activity in particular has been associated with disruptions of corneal epithelial barrier function.^{24,26}

Th-17 cells are an IL-17-producing CD4+ T-cell subset distinct from traditional Th-1 and Th-2 lineages, and have been linked to several autoimmune and autoinflammatory diseases, including DED. IL-1, IL-23, IL-17, MMP-3, and -9 are elevated in dry eye. The cytokine milieu in ocular surface in DED, rich with IL-1, IL-23, and IL-6, favors the activation of Th17 cells and production of IL-17.

IFN- γ , IL-12, IL-23, and IL-6 all signal through JAK/STAT pathways. Thus given the apparent pathogenic role of these cytokines in DED, the ability of a JAK inhibitor to block signaling of such cytokines offers the potential to modulate ocular surface inflammation in DED.

Ophthalmic Tofacitinib

Topical ophthalmic tofacitinib was developed and tested in a phase I/II clinical trial in the US in moderate to severe aqueous deficient DED patients. The safety and pharmacologic activity of tofacitinib (0.0003 % BID; 0.001 % OU BID; 0.003 % BID; 0.005 % BID; 0.005 % once daily [QD]) were examined and compared with RESTASIS[®] (BID) and placebo (vehicle) BID groups.³² In the trial, the immunomodulatory effects of tofacitinib on ocular surface inflammation in DED were also investigated with biomarker analysis using ocular specimens collected from patients.³³

Ocular epithelial cell surface expression of HLA-DR, as a biomarker for ocular surface inflammation, was measured at baseline and 8 weeks after treatment with fluorescent flow cytometry coupled with conjunctival impression cytology. Levels of HLA-DR were reduced in patients treated with tofacitinib 0.003 % BID or 0.005 % QD, compared with the vehicle or cyclosporine-treated group, indicating suppression of ocular surface inflammation in DED by tofacitinib.

Tear fluids were also collected from patients and levels of cytokines and inflammatory mediators were measured. Tear levels of IL-17, IL-1, IL-23, IL-12, IL-7, MMP-9, and MMP-3 were reduced in patients treated with tofacitinib (0.005 % once a day [SID]) for 8 weeks. Thus, in addition to modulating levels of cell surface markers of immune activation, tofacitinib also seemed to suppress tear levels of a number of proinflammatory cytokines and inflammation mediators in DED.

In a US trial, all doses of tofacitinib exhibited a reasonable safety profile and were well tolerated by patients with DED, exhibiting better patient-reported ocular tolerability than cyclosporine.³² Tofacitinib also demonstrated a trend for improving both signs and symptoms of dry eye in the study, the proportion of patients with complete corneal clearing (CCC; 100 %) at week 8 was greatest with tofacitinib 0.005 % QD (15.9 %) versus vehicle (6.7 %). Patient-reported DED symptoms, measured with the ocular surface disease index (OSDI) or ocular comfort index (OCI), showed significant improvements from baseline for all tofacitinib groups after 8 weeks of treatment, and tofacitinib demonstrated greater improvements than cyclosporine.

The tofacitinib 0.005 % QD group showed significant improvements in both a sign (Schirmer wetting without anesthesia) and symptom (OSDI environmental triggers subscale) versus vehicle and also demonstrated the highest response rate for complete corneal clearing (15.9 %) at week 8, consistent with the immunomodulatory activity observed with the tofacitinib 0.005 % QD group.

The clinical biomarker findings provide pharmacologic evidence for ophthalmic tofacitinib as a potentially effective agent for modulating ocular surface inflammation and improve ocular surface health in DED. However, the precise mechanism of action of tofacitinib in DED is not clear.

In an experimental dry eye model, topical tofacitinib treatment BID significantly decreased corneal fluorescein staining on days 12 and 15, the corneal infiltration of CD11b cells, the corneal expression of tumor necrosis factor and IL-23, and conjunctival expression of IL-17A.³⁴

Tofacitinib inhibits the JAK/STAT pathways, and tofacitinib is thought to reduce the cytokine signaling that drives ocular surface inflammation

in DED. Mechanistically, tofacitinib inhibits JAK3 thus blocking signaling of common γ c cytokines including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. It also blocks JAK1, resulting in inhibition of gp130 family including IL-6, as well as IFN- γ , IFN- α/β , and IL-10. To a lesser extent tofacitinib blocks JAK2, and therefore blocks intracellular signaling of IFN- γ and granulocyte-macrophage colony-stimulating factor (GM-CSF). Because tofacitinib blocks JAK1 and JAK2, it interferes with the signaling of IFN- γ and differentiation of IFN- γ producing Th1 cells. In DED, IFN- γ causes up-regulation of chemokine ligands, chemokine receptors, MHC class II and adhesion molecules (HLA-DR, ICAM-1, and CD40), which facilitate the infiltration of pathogenic immune cells to the ocular surface tissues exacerbating the ongoing disease process. IFN- γ also induces apoptosis of corneal epithelial cells and squamous metaplasia of conjunctival epithelia. Thus, tofacitinib could suppress Th1 T cells differentiation, infiltration of pathogenic immune cells, apoptosis, and metaplasia of ocular surface epithelia. Tofacitinib also blocks signaling of IL-23 thus inhibiting the generation of pathogenic Th17 cells and production of IL-17, as it is dependent upon IL-23. Therefore, tofacitinib could reduce levels of MMP-9 and MMP-3 and subsequent epithelial damage.

As tofacitinib blocks IL-4 and IL-21, it might be anticipated that it will interfere with the function of B cells. In addition to blocking the function of lymphocytes (adaptive immunity), tofacitinib also blocks innate immune responses. Specifically, tofacitinib blocks the effects IL-6 and INFs and thereby inhibits chemokine production. Also, because tofacitinib blocks IL-15 and IL-7, it might be anticipated that it will interfere with production of IFN- γ and IL-17 by natural killer (NK) cells and intraepithelial lymphocytes (ILCs). NK cells and ILCs are thought to be important cells that produce a large amount of inflammatory cytokines in the mucosal epithelium.

Given the apparent pathogenic role of a variety of cytokines such as IL-6, IL-12, IL-23, and IFN- γ in DED, the ability of tofacitinib to block such cytokines is likely a major aspect of their mechanism of action.

Conclusions

Topical ophthalmic tofacitinib showed pharmacologic activity in patients with DED as evidenced by its effect on inflammatory biomarkers, clinical signs, and symptoms. It could modulate ocular surface inflammation in DED through suppression of cell surface expression of immune activation molecules and production of inflammatory cytokines and mediators on the ocular surface and therefore improve ocular surface health and tear production. One possible mechanism of action is through inhibiting intracellular signaling of inflammatory cytokines including IFN- γ , IL-6, IL-12, and IL-23, thus reducing infiltration of pathogenic immune cells, production of inflammatory cytokines and MMPs, preventing apoptosis and squamous metaplasia of ocular surface epithelia, and corneal epithelial barrier breakdown. ■

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