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In Vivo Efficacy of Imatinib Mesylate, a Tyrosine Kinase Inhibitor, in the Treatment of Chemically Induced Dry Eye in Animal Models

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Purpose: Dry eye disease (DED) is a multifactorial disorder of the tears and ocular surface accompanied by ocular discomfort, visual disturbance, tear film instability, and ocular surface inflammation. In the present study, we evaluated the efficacy of the tyrosine kinase inhibitor imatinib mesylate for the treatment of DED.

Methods: Experimental models of DED were generated in Sprague Dawley rats using a combination of benzalkonium chloride (BAC) with atropine sulfate and in New Zealand White rabbits using BAC. The animals were treated twice daily with eye drops of vehicle, imatinib (0.01%–0.3%), or a positive control (Restasis). The improvement in DED due to imatinib was assessed by staining with fluorescein, lissamine green, impression cytology, and histological analysis. In addition, immunofluorescence staining was performed at the end of the study to evaluate the inflammatory response in the ocular surface.

Results: Topical application of imatinib significantly reduced ocular surface damage compared with vehicle-treated animals. Imatinib restored the morphology and structure of the conjunctival epithelium and reduced the recruitment of immune cells in the corneal epithelium. Furthermore, imatinib significantly reduced the impression cytology score, thus demonstrating that imatinib prevents the loss of goblet cells in DED animal models. The therapeutic efficacy of imatinib was similar to or better than that of cyclosporine treatment.

Conclusions: In this study, we provide an animal in vivo proof of concept of the therapeutic potential of imatinib for the treatment of DED.

Translational Relevance: With this study we show the possibility of developing imatinib as a new ophthalmic drop to treat DED.

Introduction

Dry eye disease (DED), also known as keratoconjunctivitis sicca, is the most common ophthalmic condition in the world. According to the official report of the Tear Film and Ocular Surface Society Dry Eye Workshop (DEWS 2017), DED is defined as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.[1] The prevalence of DED is continuously growing worldwide, with an incidence ranging from 9.5% to 90%, and is more prevalent in Asian countries than in Western countries.[2,3] Epidemiologic studies have determined that older age and female

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sex (particularly postmenopause) are risk factors for DED.[4,5] The prevalence of DED has continued to increase in recent years due to environmental factors such as extreme weather and reduced relative humidity, use of video display terminals, smoking, refractive surgery such as LASIK, and contact lens wear. This disease has become an inevitable public health problem because it significantly lowers the quality of life of the affected individuals.[6,7]

Among the commonly used therapies for DED, the first-line choice is topical application of artificial tears, which has been proven to relieve symptoms of irritation in mild to moderate clinical conditions by dilution of the inflammatory markers present in the tear fluid and lowering tear osmolality. However, artificial tears have no antiinflammatory properties and cannot resolve the underlying pathogenesis of DED. To date, two prescription drugs currently approved for the treatment of DED in the United States are 0.05% cyclosporine ophthalmic emulsion (Restasis; Allergan, Dublin, Ireland) and 5% lifitegrast ophthalmic solution (Xiidra; Novartis Pharmaceuticals, Basel, Switzerland). Cyclosporine suppresses inflammation by binding to cyclophilins and inhibiting calcineurin, ultimately preventing dephosphorylation of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), which in turn decreases interleukin-2 levels. Because interleukin-2 is essential for T-cell replication, cyclosporine inhibits T-cell proliferation and thereby inhibits T-cell–mediated immune responses.[8] Lifitegrast is also known to inhibit T-cell–mediated inflammation by blocking the interaction between the cell surface protein lymphocyte function-associated antigen-1 and intercellular adhesion molecule-1.[9]

Although both drugs have been shown to reduce DED symptoms in patients, a relatively large percentage of patients have been reported to have an incomplete response to drugs or have a significant incidence of adverse effects, mostly instillation irritation, but also ocular pain, dysgeusia, and decreased visual acuity.[10,11] Thus, despite recent developments in the management of DED, new therapeutic approaches that can improve the ocular surface microenvironment are urgently needed.

Imatinib mesylate (STI571, Gleevec; Novartis Pharmaceuticals) is a tyrosine kinase inhibitor (TKI) that was originally approved for the treatment of chronic myeloid leukemia in the first-line setting. Imatinib mesylate (imatinib) was also repurposed within the wider disease subtype (for example, acute lymphoblastic leukemia and myelodysplastic diseases) than for which it was initially approved. Because imatinib showed cross-reactivity and ability to inhibit c-Kit receptor tyrosine, which has been identified as a cause of gastrointestinal stromal tumors, it was subsequently approved by the US Food and Drug Administration (FDA) for gastrointestinal stromal tumor treatment. Likewise, as imatinib also cross-reacts with platelet-derived growth factor receptor kinase, it has been used for the treatment of dermatofibrosarcoma and systemic mastocytosis.[12] Because its safety data already exist, repurposing of imatinib has a great advantage in the accelerated drug development process. There have been numerous studies on the application of imatinib for other indications, such as ischemia/reperfusion injury lung diseases, viral diseases, vascular leak, and rheumatoid arthritis.[13–16]

As a kinase inhibitor, imatinib has also been shown to have potent antiinflammatory effects by modulating proinflammatory cytokine production, as well as monocyte/macrophage activation, in numerous animal models of autoimmune diseases.[17] Among the receptor tyrosine kinases involved in inflammatory disorders, discoidin domain receptor 1 (DDR1), a class of collagen-activated receptor tyrosine kinase, has been identified as a potential target of imatinib.[18,19] It has also been reported that DDR1 is widely expressed in epithelial cells, including cornea, lung, kidney, colon, and brain tissues.[18,20] These findings suggest that imatinib may possess potent antiinflammatory activity targeting DDR1 in the eye; therefore, imatinib could be suitable for treating DED. A study by Hagan et al.[21] reported that TOP2362, a narrow-spectrum kinase inhibitor, showed therapeutic efficacy in the treatment of DED by modulating protein kinases and attenuating inflammation. This further supports our hypothesis that protein kinase inhibitors may have potential in the treatment of DED. We hypothesized that imatinib may reduce the inflammatory response associated with DED. In this study, we investigated the effect of imatinib on chemically induced DED animal models of Sprague Dawley rats and New Zealand White rabbits.

Methods

Reagents

Imatinib mesylate (hereafter imatinib) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Isopto atropine eye drop (1% atropine sulfate) was obtained from Alcon Laboratories (Ft. Worth, TX). Benzalkonium chloride (BAC) and other standard reagents were purchased from Sigma-Aldrich (St. Louis, MO). Primary antibodies for lymphocyte-specific protein 1 (LSP1) and F4/80 were purchased from Abcam (Cambridge, UK), and DDR-1 was

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obtained from Novus Biologicals (Littleton, CO). The secondary antibody used in this study, goat anti-rabbit IgG antibody conjugated with tetramethylrhodamine isothiocyanate, was purchased from Santa Cruz Biotechnology (Dallas, TX).

Efficacy Evaluation in Rats

Eight-week-old male Sprague Dawley rats were purchased from Orient Bio (Seongnam, Korea). Rats were housed in an animal care facility, provided with food and water ad libitum, and exposed to a 12/12-hour light/dark cycle at 23°C ± 3°C. All experimental procedures were approved by the institutional animal care and use committee of the School of Medicine and the animal research ethics committee of the Catholic University of Korea and complied with the Laboratory Animals Welfare Act, in accordance with the *Guide for the Care and Use of Laboratory Animals* (IACUC approval no. CUMC-2014-0194-01).

Keratoconjunctivitis sicca was induced by administering eye drops containing 0.5% BAC in 1% atropine sulfate, twice daily for 2 weeks. Imatinib was firstly dissolved in balanced salt solution (1 mg/mL), further diluted prior to administration, and applied dropwise in the eye twice a day for 1 week after 7 days of administration of 0.5% BAC in 1% atropine sulfate. To compare the therapeutic effects, 0.05% cyclosporine A (CsA; Restasis) was used as the reference drug.

Efficacy Evaluation in Rabbits

Twenty-five male New Zealand White rabbits (Hallym Laboratory of Experimental Animals, Hwaseong, Korea), weighing between 2 and 2.5 kg, were used for this study. The rabbits were quarantined and acclimatized for a week before the experiments. They were housed separately in stainless steel cages (500 mm wide × 800 mm long × 500 mm high) in an environmentally controlled room (temperature, 23°C ± 3°C; relative humidity, 55% ± 15%; 12/12-hour light/dark cycle of 150–300 Lux; ventilation, 10–20 times per hour). Food and sterilized water were available ad libitum. The animal study was approved by the institutional animal care and use committee at KNOTUS Co. Ltd. (Guri-si, Korea; approval no. KNOTUS IACUC 17-KE-360).

To induce keratoconjunctivitis sicca, 0.5% BAC was administered topically, once daily for 14 days, in both eyes of the rabbits. Rabbits with dry eye condition were then randomly divided into five groups (five per group): vehicle (phosphate buffered saline [PBS])-treated, 0.05% imatinib-treated, 0.1% imatinib-treated, 0.3% imatinib-treated, and 0.05% CsA-treated. The positive control was 0.05% CsA eye drops (T-Sporin; Hanlim Pharm. Co. Ltd., Seoul, Korea). After induction of dry eye, on the 14th day following initial BAC topical administration, imatinib and CsA were prepared according to the dose, and animals were administered 50 µL of the respective eye drops, two times daily for 14 days. All animals were closely monitored, and no clinical symptoms were observed during the entire experimental period.

Corneal Fluorescein Staining

Corneal surface staining was performed using fluorescein solution. Briefly, two drops of 1% fluorescein (approximately 70 µL) was applied consecutively to the conjunctival sac of the rat eye, the excess fluorescein solution was removed with a cotton swab, and it was then washed three times with PBS. Fluorescence imaging of stained corneas was performed using an OPMI 1 FR pro microscope (Carl Zeiss Microscopy, Jena, Germany). To analyze fluorescein staining in rabbit, 50 µL of 2% fluorescein was dropped into the conjunctival sac of both eyes with a micropipette and the excess fluorescein solution was removed with gauze. Ocular surface staining was then evaluated using a slit lamp under a cobalt blue light. Images were captured by a TRC-50IX retinal camera (Topcon, Tokyo, Japan). All of the images were acquired using the same intensity settings. Images were analyzed using ImageJ (National Institutes of Health, Bethesda, MD), and the percentage of fluorescein-stained area was calculated.

Corneal Dye Staining

To evaluate changes in the corneal epithelial cells, one eye of each rat was stained with 1% lissamine green (Sigma-Aldrich). Images of lissamine green staining on the corneal surface were taken using the Zeiss OPMI 1 FR pro microscope and then analyzed using ImageJ. The values were calculated as the percentage of the total area and punctuate staining area.

Immunofluorescence Microscopy

The tissue sections were first incubated with primary antibodies against LSP1 (to assess leukocyte migration in inflammation), F4/80 (for macrophage visualization), and DDR1, followed by secondary fluorescent antibodies, according to the origin of the primary antibodies. Rhodamine phalloidin (Invitrogen, Carlsbad, CA) was used to visualize filamentous (F)-actin. Nuclei were stained with 4′,6-diamidino-2-phenylindole. Images were analyzed using an Axio

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Imager M2 microscope (Carl Zeiss Microscopy), and the number of F4/80-positive and LSP1-positive cells was counted per microscopic field.

Histopathological Analysis

Eyes were enucleated at the end of the study, fixed in 4% paraformaldehyde, and then placed in 10% normal buffered formalin. Tissues were then processed and embedded in paraffin for sectioning and stained with hematoxylin and eosin (H&E). The sections were imaged using an Olympus BX53 microscope (Olympus, Tokyo, Japan). Inflammatory and fibrosis indices were evaluated as previously described.[22] The criteria for inflammation and fibrosis scoring based on H&E staining results were as follows: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms.

Conjunctival Impression Cytology

At the end of the study, specimens were obtained for impression cytology from the eyes of the animals to determine the density and changes in conjunctival goblet cells. Small disks of nitrocellulose filter paper (Pall Corporation, New York, NY) were cut into pieces (3.5 mm × 3.5 mm in size), applied separately on the nasal and temporal bulbar conjunctiva areas, and pressed for 10 seconds with constant pressure. Cells adhering to the membrane were fixed and stained with periodic acid–Schiff (PAS). The goblet cells were stained red using PAS under the microscope. The cell morphology was viewed, and images were obtained using light microscopy (Olympus). Five images (two superior, two inferior, and one temporal) per eye of each rabbit were analyzed. All specimens were graded according to Tseng's classification.[23] The criteria for scoring were as follows: 0, normal; 1, early loss of goblet cells; 2, total loss of goblet cells; 3, early keratinization; 4, moderate keratinization; and 5, advanced keratinization.

Statistical Analysis

The results obtained in this study are expressed as mean ± standard deviation. Statistical analysis was performed with Prism (GraphPad Software, San Diego, CA) using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. For corneal fluorescein imaging of rabbits, the test was performed using one-way ANOVA with Dunnett's multiple comparison test. Statistical significance was defined as $P < 0.05$.

Results

Effect of Imatinib on Ocular Surface Damage in the BAC-Induced DED Rat Model

The aim of this study was to determine whether imatinib has therapeutic potential against DED through the use of an in vivo model. Because atropine, a muscarinic receptor antagonist, is known to decrease tear volume and alter tear film stability, in order to ensure establishment of an aqueous tear deficiency model, we used atropine with BAC, an agent widely use to induce DED in animals.[24]

To assess the epithelial integrity of the ocular surface, staining of the cornea using fluorescein and lissamine green was performed. The fluorescein staining test is a useful and common tool to evaluate corneal epithelial damage in patients with DED because it can provide important information regarding the level of tissue damage.[25] As shown in Figure 1A, after 7 days of treatment with BAC containing atropine, the area of the cornea stained with fluorescein was observed in the DED model group. The corneal fluorescein-stained area was significantly reduced in rats treated with the different doses of imatinib, in a dose-dependent manner (Fig. 1A). The reduction in fluorescein staining area was almost maximum for 0.1% imatinib. Based on this result, we used 0.1% imatinib in subsequent experiments in rats. In addition, lissamine green staining of the ocular surface was performed to detect keratinized and devitalized epithelial cells on the ocular surface.[26] Representative images of lissamine green staining are shown in Figure 1B. In this study, 0.05% CsA, a widely used drug for the treatment of dry eye, was used as a positive reference compound. The normal group did not exhibit any detectable lissamine green staining. After 7 days of treatment with 0.1% imatinib, the area stained with lissamine green was reduced compared with that in the DED (vehicle) group (2.56% ± 0.39% vs. 10.90% ± 1.26%; $^{***}P < 0.001$; $n = 3$ per group) (Fig. 1B).

Effect of Imatinib on Corneal Inflammation in the BAC-Induced DED Rat Model

Treatment with BAC, which induces DED through corneal damage, has been reported to cause ocular discomfort, loss of goblet cells, inflammation, conjunctival squamous metaplasia, epithelial cell apoptosis, and subconjunctival fibrosis.[27] To evaluate the effect of imatinib on BAC-induced epithelial integrity in the cornea, we performed immunofluorescence staining using rhodamine–phalloidin, which recognizes

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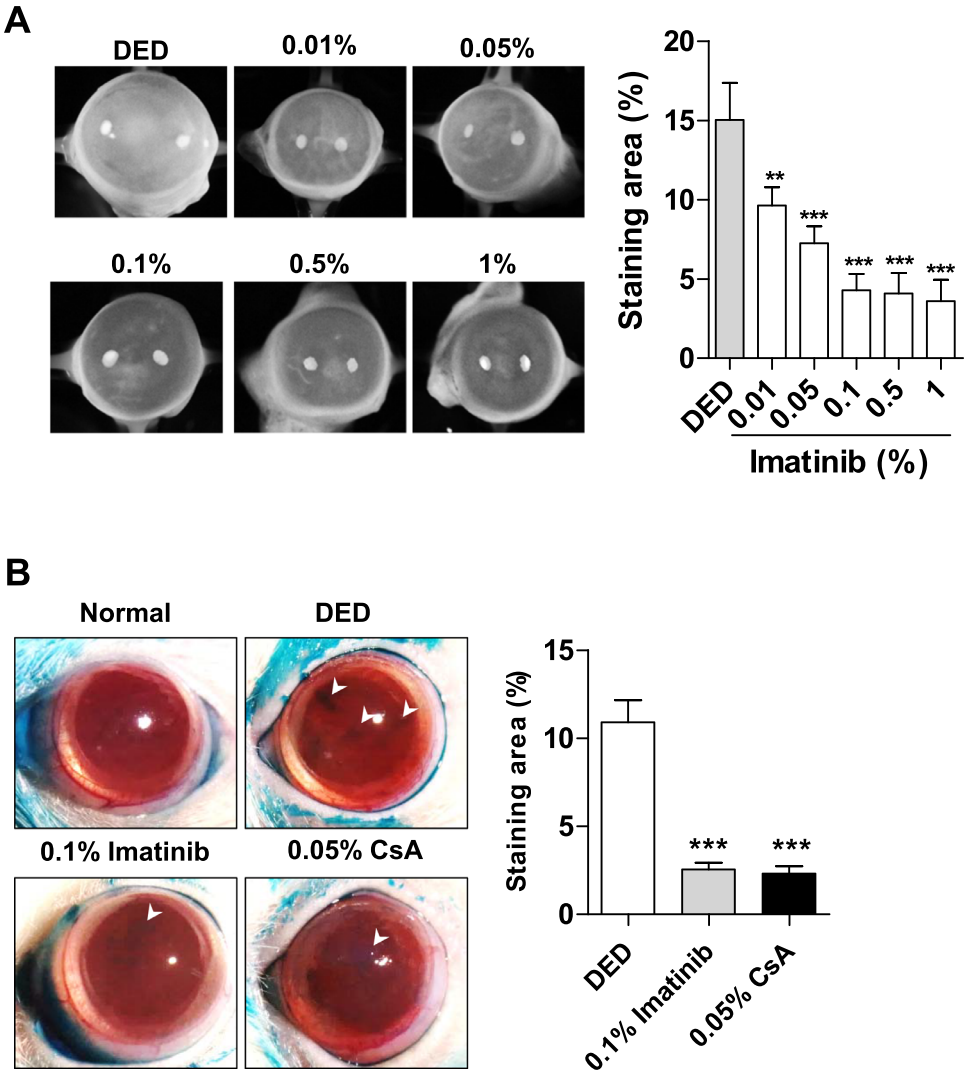

Figure 1. Alterations of the ocular surface after imatinib treatment. (**A**) Corneal fluorescein staining areas (%) for the various doses of imatinib treatment in rats. (**B**) Representative images of corneal lissamine green staining (*arrowheads*). The *right panel* shows quantification of the corneal lissamine green staining. The data are expressed as mean ± standard deviation ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$ versus DED group.

F-actin. In the BAC-induced DED model group, F-actin–positive cells were apparently present on the ocular surface, whereas F-actin–positive cells were sparse in the eyes of the imatinib-treated group, as was the case in the 0.05% CsA-treated group (Fig. 2A).

Inflammation is critical in DED pathogenesis and progression and is involved in the recruitment of inflammatory immune cells to the ocular surface, where they secrete proinflammatory mediators.[28,29] To elucidate whether imatinib improves DED-associated inflammation by suppressing the recruitment of inflammatory cells, immunofluorescence staining was performed. We detected LSP1, which is a specific marker for leukocytes and F4/80, which is considered one of the key markers of activated macrophages in rat corneal tissue. The number of F4/80-positive and LSP1-positive cells increased in the DED group compared with that in the normal group. In contrast, treatment with 0.1% imatinib significantly reduced the accumulation of F4/80-positive cells and LSP1-positive cells in the cornea (Figs. 2B, 2C).

DDR1 is a family of two non-integrin collagen receptors that display tyrosine kinase activity and promote inflammation.[18,19] Because previous studies have reported that DDR1 is expressed in the cornea and imatinib targets DDR1,[18,30] we next

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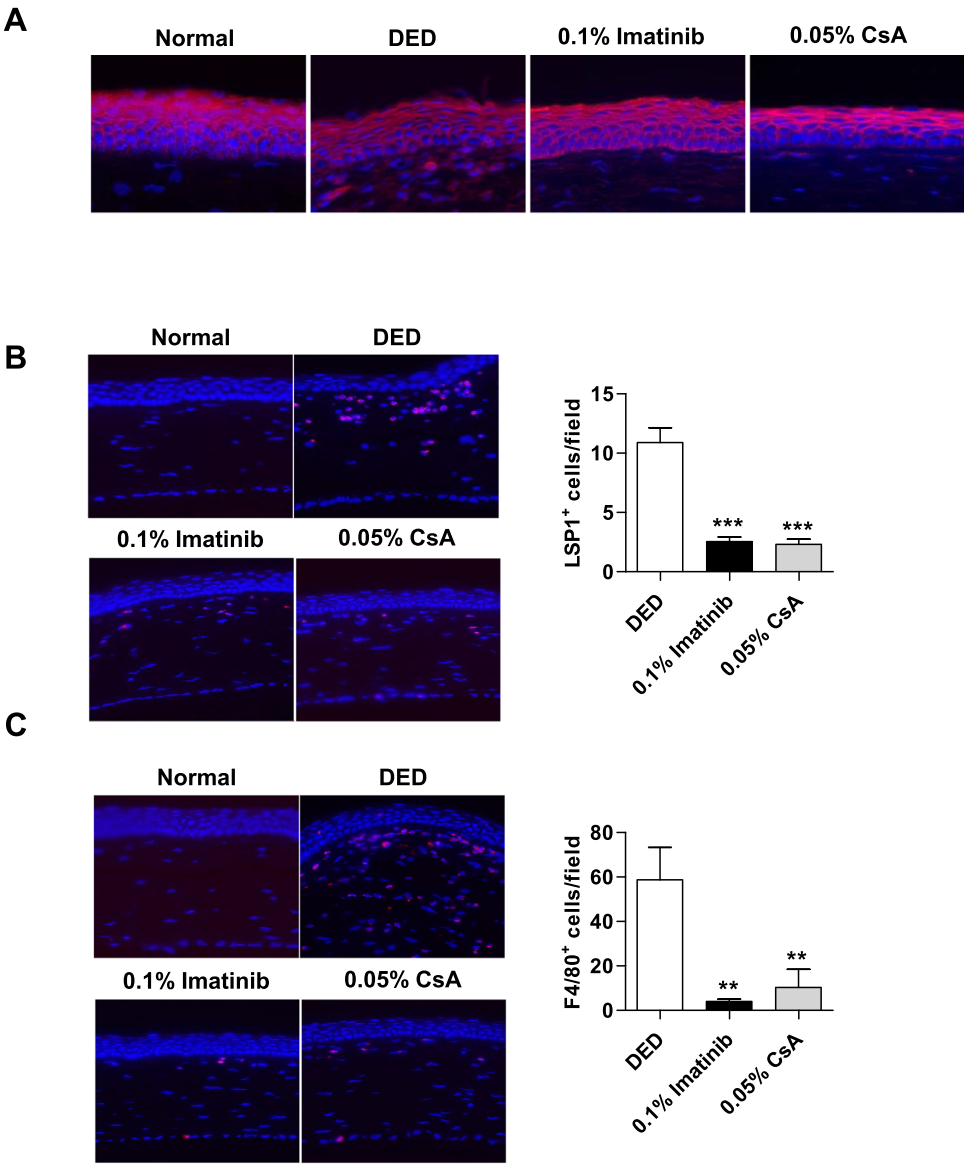

Figure 2. Effect of imatinib eye drops on epithelium integrity and inflammatory cell infiltration. (**A**) F-actin staining was applied to measure cornea epithelium integrity. (**B**) Immunofluorescence images and LSP1-positive leukocyte counts. (**C**) Immunofluorescence images and F4/80-positive macrophage counts in rat eyes. The data are expressed as mean ± standard deviation ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$ versus DED group.

evaluated whether imatinib exerts antiinflammatory effects through DDR1 regulation in a BAC-induced DED model. As shown in Figure 3, the expression of DDR1 (pinkish-red color) in the cornea was higher in the DED group than in the normal control group. However, the intensity of DDR1 expression in the imatinib-treated group was weaker than that in the DED group (Fig. 3). These results demonstrate the beneficial effect of topical application of imatinib in ameliorating ocular damage and reversing inflammatory changes in DED.

Effect of Imatinib on Corneal Damage in the DED Rabbit Model

The murine model allows evaluation of the efficacy and mechanisms of drug candidates at a relatively low cost. However, it has some limitations in its use in drug development, especially with regard to the treatment of ophthalmologic diseases, in terms of physiology and anatomy. On the other hand, the DED rabbit model has several advantages, including globe size, well-defined eye anatomy, histology, and that the expression levels

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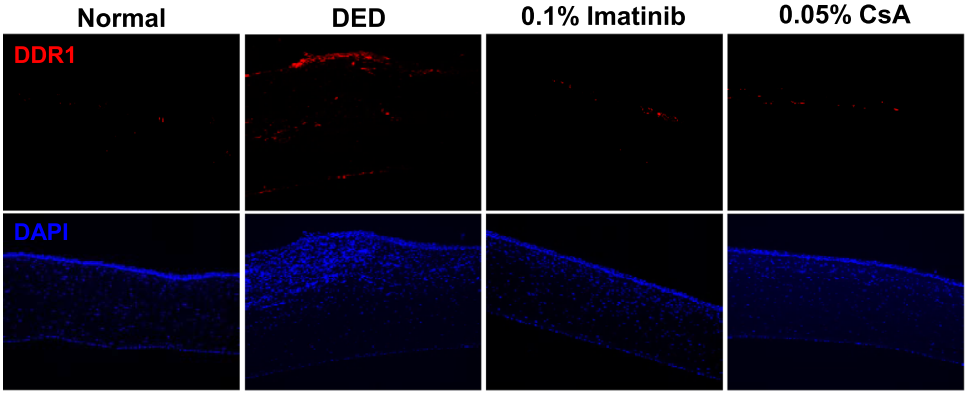

Figure 3. Imatinib eye drops reduced the expression of DDR1 in the dry eye rat model. The expression of DDR1 increased in the DED group but was significantly suppressed by imatinib ($n = 3$).

of enzymes involved in drug metabolism in rabbits are much closer to those of human tissue than in murine models. Therefore, we assessed the therapeutic efficacy of imatinib in a BAC-induced rabbit DED model.

To investigate the effect of imatinib on corneal damage in experimental dry eye, imatinib was delivered in the form of eye drops to rabbits, and corneal fluorescein staining was conducted. As shown in Figure 4A, topical application of imatinib for 14 days remarkably reduced the fluorescein-stained area in a dose-dependent manner compared with that in the untreated DED group. Corneal staining scores were also evaluated. Although the staining scores of imatinib decreased in a dose-dependent manner, the differences were not statistically significant.

There is a growing body of evidence suggesting that dry eye is accompanied by inflammation, and inflammatory conditions are closely associated with fibrosis.[31] We next determined whether imatinib has an effect on the histopathological changes induced by BAC. As shown in Figure 4B, representative images of H&E-stained corneas showed that BAC induced histological inflammation, including immune cell infiltration, whereas the topical application of imatinib mitigated inflammation on the ocular surface. In comparison with the untreated DED group, imatinib also elicited dose-dependent decreases in fibrosis scores, concomitant with reductions in inflammation scores (Fig. 4B).

Effect of Imatinib on Conjunctival Goblet Cell Protection

The main role of goblet cells is to produce and secrete mucins that lubricate the ocular surface, and a decrease in the number of goblet cells is one of the major etiologies of DED. Therefore, the presence and number of goblet cells play a crucial role in the understanding and diagnosis of ocular diseases.[32] To investigate whether topical application of imatinib exhibits protective effects on goblet cells after BAC treatment, PAS staining was performed to analyze the number and morphology of goblet cells in the conjunctiva. PAS stains neutral mucin; therefore, the cytoplasm of goblet cells, whose primary function is to secrete mucin, is strongly positive for PAS. As shown in Figure 5A, the morphology of goblet cells in the BAC-induced DED group was small and shriveled. On the other hand, we observed that the intensity of the PAS-positive cells (pinkish-red color) increased in a dose-dependent manner in the imatinib-treated groups compared with the vehicle group. Further, the changes were evaluated by scoring. The mean scores for the 0.1% topical imatinib group (2.60 ± 0.23) and for the 0.3% topical imatinib group (2.45 ± 0.57) were significantly lower than those of the DED group (3.63 ± 0.26) (Fig. 5B). Moreover, it should be noted that the score of the 0.3% imatinib-treated group (2.45 ± 0.57; $^{***}P < 0.001$ versus DED group) was lower than that of the CsA-treated group (2.70 ± 0.20; $^{**}P < 0.01$ versus DED group), suggesting that imatinib protected goblet cells to reduce loss and the effect was even better than that of 0.05% CsA.

Discussion

In the present study, we evaluated the efficacy of imatinib, an FDA-approved TKI, in murine and rabbit experimental models of DED. We also aimed to determine whether the beneficial effect against DED is observed at imatinib doses that are substantially lower than the levels used for cancer treatment. In addition,

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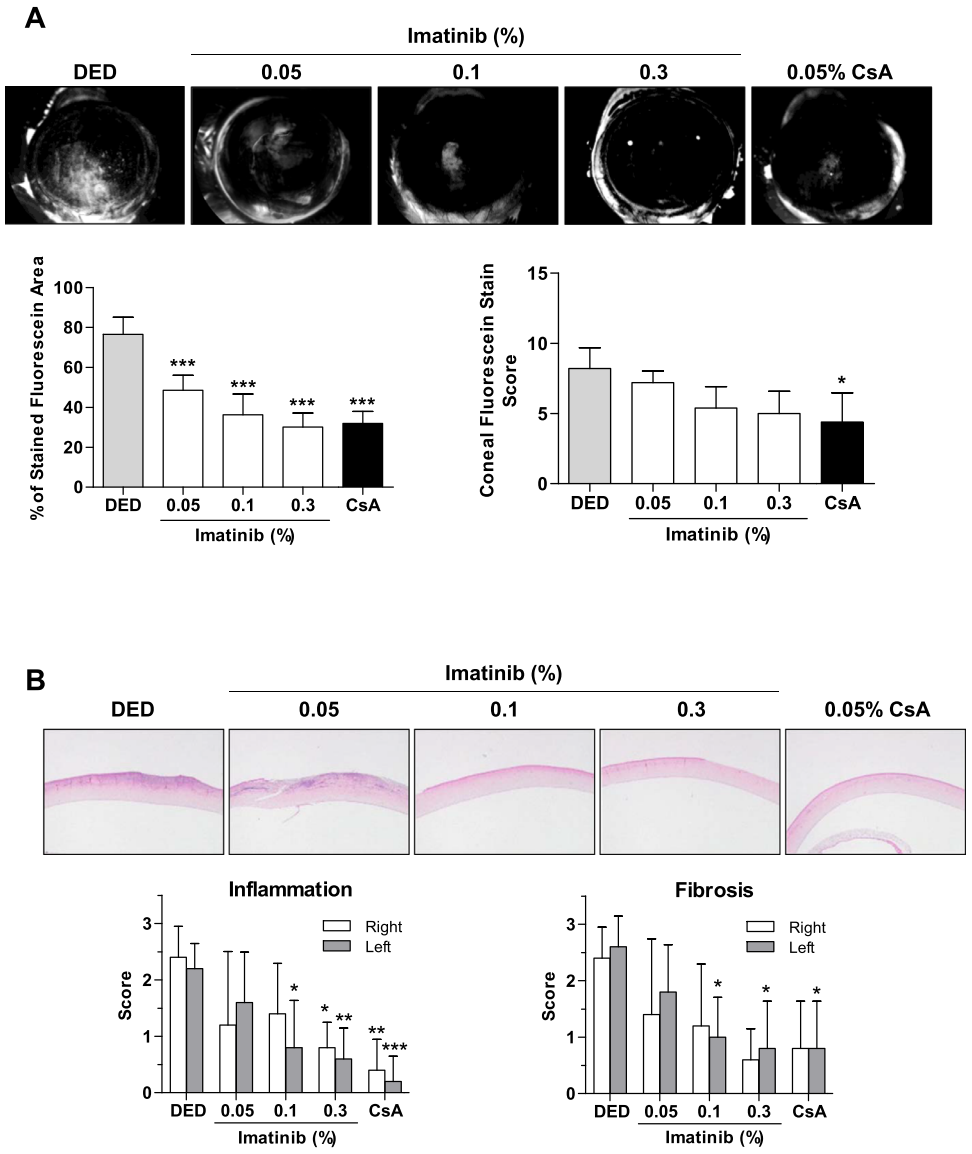

Figure 4. Protective effects of imatinib on the corneas of rabbit with BAC-induced DED. (**A**) Representative photographs of corneal fluorescein staining and corneal fluorescein staining area. (**B**) Representative images of H&E staining and inflammation and fibrosis scores. The data are expressed as mean ± standard deviation ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus DED group.

we compared the efficacy of imatinib with 0.05% CsA, a commercially available drug for treating dry eye. Our results show that twice-daily ocular administration of imatinib provided therapeutic benefit against ocular surface damage associated with DED, an efficacy that was equivalent to or better than that of CsA. In addition to reducing corneal surface damage, imatinib also alleviated inflammation and reversed the loss of goblet cells in a BAC-induced DED animal model. Taking into consideration these data, it was concluded that imatinib is a promising clinical therapeutic agent for the treatment of DED.

It is well recognized that inflammation plays an etiological role in DED, disrupts homeostasis, and causes injury to the ocular surface. Thus, hyperosmotic stress, a common pathogenic mechanism of DED, can trigger a vicious cycle of inflammation, leading to further ocular surface injury. As a result, the levels of proinflammatory mediators such as tumor necrosis factor-α, IL-1, IL-6, and nuclear factor-κB increase, resulting in the recruitment of inflammatory cells.[33] Because the end point of decreased tear secretion and tear osmolality changes is inflammation in the ocular surface, the present study focused primarily on the antiinflammatory effect of imatinib in DED. The topical application of 0.1% imatinib was found to exhibit effective antiinflammatory activity, as evidenced by the significant decrease in the recruitment

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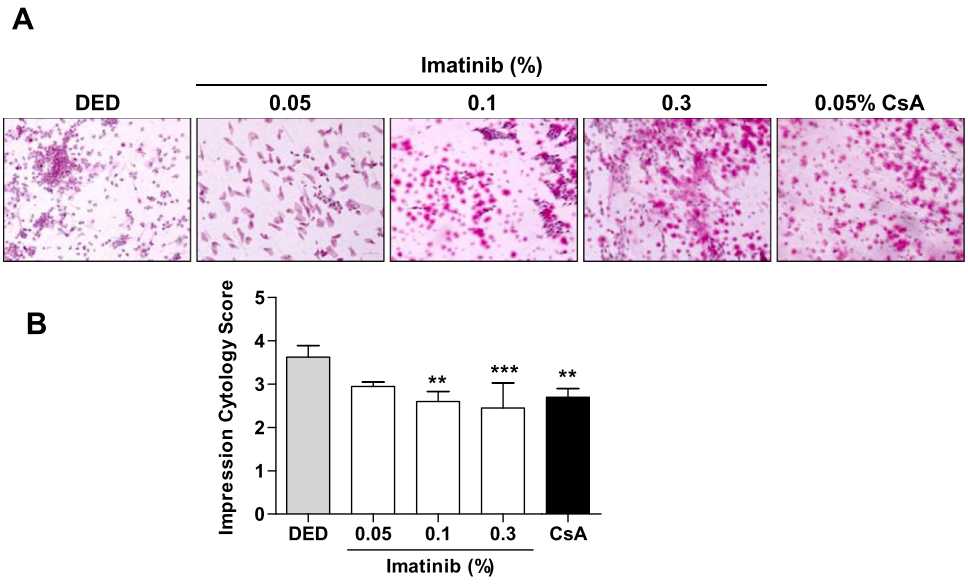

Figure 5. (**A**) Alterations of goblet cells in conjunctiva observed by PAS staining. (**B**) Representative image of impression cytology and score. The data are expressed as mean ± standard deviation ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$ versus DED group.

of macrophages and leukocytes, which are the major source of inflammatory mediators in a rat model of DED. Several reports have shown that imatinib exhibits therapeutic effects via the suppression of inflammatory cytokines and chemokines in acute hepatitis, liver fibrosis arthritis, and chronic myocarditis.[34–37] Our findings are consistent with those of the previous studies and suggest that the antiinflammatory effect of imatinib results in an improvement in dry eye symptoms.

In the present study, we also found that the level of DDR1 in the ocular surface of BAC-induced rats was elevated, and topical application of imatinib suppressed DDR1 expression. The discoidin domain receptors, DDR1 and DDR2, are non-integrin collagen receptors that contain a discoidin homology domain in their extracellular regions and are members of the receptor tyrosine kinase family. Both DDRs bind to a number of different collagen types and play important roles in embryo development. Upon collagen binding, DDRs transduce cellular signaling involved in various cellular functions, such as cell adhesion, proliferation, differentiation, migration, and matrix homeostasis. Dysregulation of DDR is associated with the progression of a variety of diseases, including fibrosis, arthritis, atherosclerosis, inflammation, and cancer.[30] Mohan et al.[18] reported the expression of DDRs in human corneal tissue. The precise roles of DDR1 and DDR2 in the cornea remain poorly understood; however, it seems possible that collagen within epithelial or endothelial basement membranes or the stoma of connective tissues of the cornea may mediate trophic effects in the associated cells via DDR1 and DDR2.[18] Thus, DDR1 is considered an attractive target for drug discovery. A study using a chemical proteomics approach reported that the clinically approved BCR-ABL kinase inhibitors, such as imatinib, nilotinib, and dasatinib, are also potent inhibitors of DDR1 and DDR2.[38] There are reports that the overexpression of receptor tyrosine kinases results in elevated receptor tyrosine kinase signaling, and dimerization and/or activation of DDR is ligand independent.[39–41] These reports, in part, support our hypothesis that imatinib improves DED by regulating inflammatory response through inhibition of DDR1 expression. Indeed, until recently, little information existed on the transcriptional regulation of DDR1. The Ras/Raf/ERK pathway has been reported to be involved in the regulation of DDR1 transcription.[42,43] Ruiz and Jarai[43] reported that DDR1 expression in primary lung fibroblasts can be increased by collagen I, a ligand for both DDR1 and DDR2, through DDR2 activation, in an ERK1/2-dependent manner. Moreover, DDR1 itself can positively regulate its own expression through the activation of the Ras/Raf/ERK pathway in DDR1 upregulation.[42] However, the detailed molecular mechanisms that regulate DDR1 transcription have not yet been defined. Future studies on the molecular mechanisms of imatinib on DDR1 modulation, including activation and expression levels in corneal disorders, remain to be investigated.

Our results showed a decreased overall conjunctival impression cytology score in the DED-induced group receiving imatinib compared with that in the DED group, with a dose–response relationship. Goblet cells

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within the conjunctival epithelium are highly specialized cells that synthesize, store, and secrete mucins on the surface of the eye. It is known that inflammation in dry eye conditions can lower the number of these cells,[32] which was also observed in the present study. The number of goblet cells is believed to be directly related to mucin secretion, which is attributed to lubrication of the ocular surface and prevention of ocular surface damage leading to an inflammatory response. Hence, the reduction in inflammatory cell infiltration and histological signs of inflammation may be the result of goblet cell protection by imatinib. Whether or not imatinib promotes mucin production and the underlying mechanisms by which it might have an effect were not explored in the present study, and further investigation is needed to clarify these points.

Previous studies have reported that systemic anticancer therapies with TKIs cause ocular complications, including acute and chronic damage to the eye.[44] Interestingly, however, several TKIs, including human epidermal growth factor receptor 2 inhibitors and epidermal growth factor receptor inhibitors, have been reported to cause ophthalmic side effects, such as dry eye syndrome, whereas the two common ocular side-effects related to imatinib are periorbital edema and epiphora.[45] In this regard, it should be noted that the beneficial effect on DED in animal models can be obtained with a low dose of imatinib compared with the human equivalent dose that is currently used for anti-cancer treatment. The standard dose of imatinib for the treatment of chronic-phase chronic myeloid leukemia is 400 mg/day; therefore, the highest doses administered to rats (0.1%) and rabbits (0.3%) in our study were equivalent to 2.9 to ~6.5 µg/day in a human adult, based on guidelines of the FDA. These doses are substantially lower than those used for treating chronic myeloid leukemia and other cancers (typically 400–800 mg/day).[46] Based on the results obtained for 0.3% imatinib topical application in our ongoing phase I clinical trial in Korea, the plasma level for imatinib was 0.035% that of the plasma concentration for oral administration in cancer patients (manuscript in preparation). In the present study, no significant adverse effects or signs of toxicity were observed during the experimental period with imatinib in either rats or rabbits.

Conclusions

We found that imatinib administration showed clinical improvements by maintaining the integrity of the corneal surface and ameliorating ocular surface inflammation in an animal model of DED. To our knowledge, this is the first study to report that the topical application of imatinib has great therapeutic potential for the treatment of DED. The efficacy of imatinib for the treatment of ocular inflammation and corneal damage was similar to that of the available prescription drug Restasis, which is a CsA emulsion; however, protection of goblet cells was even better than that of CsA. Currently, the safety and pharmacokinetics of imatinib eye drops in human have been successfully evaluated in a phase I placebo-controlled, randomized, double-blind trial in Korea. Our findings provide new insights into the therapeutic potential of imatinib in dry eye disease.

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References

1. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II Definition and Classification report. *Ocul Surf*. 2017;15(3):276–283.
2. Shanti Y, Shehada R, Bakkar MM, Qaddumi J. Prevalence and associated risk factors of dry eye disease in 16 northern West Bank towns in Palestine: a cross-sectional study. *BMC Ophthalmol.* 2020;20(1):26.
3. Um SB, Kim NH, Lee HK, Song JS, Kim HC. Spatial epidemiology of dry eye disease: findings from South Korea. *Int J Health Geogr.* 2014;13:31.
4. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II Epidemiology report. *Ocul Surf.* 2017;15(3):334–365.

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5. Malet F, Le Goff M, Colin J, et al. Dry eye disease in French elderly subjects: the Alienor Study. *Acta Ophthalmol.* 2014;92(6):e429–436.
6. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. *Clin Ophthalmol.* 2009;3:405–412.
7. Epidemiology DEWS Subcommittee. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* 2007;5(2):93–107.
8. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. *Immunopharmacology.* 2000;47(2-3):119–125.
9. Perez VL, Pflugfelder SC, Zhang S, Shojaei A, Haque R. Lifitegrast, a novel integrin antagonist for treatment of dry eye disease. *Ocul Surf.* 2016;14(2):207–215.
10. Jones L, Downie LE, Korb D, et al. TFOS DEWS II Management and Therapy report. *Ocul Surf.* 2017;15(3):575–628.
11. Donnenfeld ED, Karpecki PM, Majmudar PA, et al. Safety of lifitegrast ophthalmic solution 5.0% in patients with dry eye disease: a 1-year, multicenter, randomized, placebo-controlled study. *Cornea.* 2016;35(6):741–748.
12. Iqbal N, Iqbal N. Imatinib: a breakthrough of targeted therapy in cancer. *Chemother Res Pract.* 2014;2014:357027.
13. El Bairi K, Trapani D, Petrillo A, et al. Repurposing anticancer drugs for the management of COVID-19. *Eur J Cancer.* 2020;141:40–61.
14. Eklund KK, Joensuu H. Treatment of rheumatoid arthritis with imatinib mesylate: clinical improvement in three refractory cases. *Ann Med.* 2003;35(5):362–367.
15. Rizzo AN, Aman J, van Nieuw Amerongen GP, Dudek SM. Targeting Abl kinases to regulate vascular leak during sepsis and acute respiratory distress syndrome. *Arterioscler Thromb Vasc Biol.* 2015;35(5):1071–1079.
16. Wahlgren N, Thoren M, Hojeberg B, et al. Randomized assessment of imatinib in patients with acute ischaemic stroke treated with intravenous thrombolysis. *J Intern Med.* 2017;281(3):273–283.
17. Azizi G, Mirshafiey A. Imatinib mesylate: an innovation in treatment of autoimmune diseases. *Recent Pat Inflamm Allergy Drug Discov.* 2013;7(3):259–267.
18. Mohan RR, Mohan RR, Wilson SE. Discoidin domain receptor (DDR) 1 and 2: collagen-activated tyrosine kinase receptors in the cornea. *Exp Eye Res.* 2001;72(1):87–92.
19. Borza CM, Pozzi A. Discoidin domain receptors in disease. *Matrix Biol.* 2014;34:185–192.
20. Li Y, Lu X, Ren X, Ding K. Small molecule discoidin domain receptor kinase inhibitors and potential medical applications. *J Med Chem.* 2015;58(8):3287–3301.
21. Hagan S, Fyfe MCT, Ofori-Frimpong B, et al. Narrow spectrum kinase inhibitors demonstrate promise for the treatment of dry eye disease and other ocular inflammatory disorders. *Invest Ophthalmol Vis Sci.* 2018;59(3):1443–1453.
22. Parrilha LR, Nai GA, Giuffrida R, et al. Comparison of 1% cyclosporine eye drops in olive oil and in linseed oil to treat experimentally-induced keratoconjunctivitis sicca in rabbits. *Arq Bras Oftalmol.* 2015;78(5):295–299.
23. Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology.* 1985;92(6):728–733.
24. Burgalassi S, Panichi L, Chetoni P, Saettone MF, Boldrini E. Development of a simple dry eye model in the albino rabbit and evaluation of some tear substitutes. *Ophthalmic Res.* 1999;31(3):229–235.
25. Barabino S, Dana MR. Animal models of dry eye: a critical assessment of opportunities and limitations. *Invest Ophthalmol Vis Sci.* 2004;45(6):1641–1646.
26. Whitcher JP. Clinical diagnosis of the dry eye. *Int Ophthalmol Clin.* 1987;27(1):7–24.
27. Baudouin C, Labbe A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. *Prog Retin Eye Res.* 2010;29(4):312–334.
28. Villani E, Baudouin C, Efron N, et al. In vivo confocal microscopy of the ocular surface: from bench to bedside. *Curr Eye Res.* 2014;39(3):213–231.
29. Villani E, Mantelli F, Nucci P. In-vivo confocal microscopy of the ocular surface: ocular allergy and dry eye. *Curr Opin Allergy Clin Immunol.* 2013;13(5):569–576.
30. Leitinger B. Discoidin domain receptor functions in physiological and pathological conditions. *Int Rev Cell Mol Biol.* 2014;310:39–87.
31. Yamaguchi T. Inflammatory response in dry eye. *Invest Ophthalmol Vis Sci.* 2018;59(14):DES192–DES199.
32. Dartt DA, Masli S. Conjunctival epithelial and goblet cell function in chronic inflammation and ocular allergic inflammation. *Curr Opin Allergy Clin Immunol.* 2014;14(5):464–470.
33. Acera A, Rocha G, Vecino E, Lema I, Duran JA. Inflammatory markers in the tears of patients with ocular surface disease. *Ophthalmic Res.* 2008;40(6):315–321.
34. Wolf AM, Wolf D, Rumpold H, et al. The kinase inhibitor imatinib mesylate inhibits TNF-{alpha}

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production in vitro and prevents TNF-dependent acute hepatic inflammation. *Proc Natl Acad Sci USA.* 2005;102(38):13622–13627.
35. Yoshiji H, Noguchi R, Kuriyama S, et al. Imatinib mesylate (STI-571) attenuates liver fibrosis development in rats. *Am J Physiol Gastrointest Liver Physiol.* 2005;288(5):G907–G913.
36. Leipner C, Grun K, Muller A, et al. Imatinib mesylate attenuates fibrosis in coxsackievirus b3-induced chronic myocarditis. *Cardiovasc Res.* 2008;79(1):118–126.
37. Akashi N, Matsumoto I, Tanaka Y, et al. Comparative suppressive effects of tyrosine kinase inhibitors imatinib and nilotinib in models of autoimmune arthritis. *Mod Rheumatol.* 2011;21(3):267–275.
38. Day E, Waters B, Spiegel K, et al. Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. *Eur J Pharmacol.* 2008;599(1–3):44–53.
39. Noordeen NA, Carafoli F, Hohenester E, Horton MA, Leitinger B. A transmembrane leucine zipper is required for activation of the dimeric receptor tyrosine kinase DDR1. *J Biol Chem.* 2006;281(32):22744–22751.
40. Abdulhussein R, Koo DH, Vogel WF. Identification of disulfide-linked dimers of the receptor tyrosine kinase DDR1. *J Biol Chem.* 2008;283(18):12026–12033.
41. Du Z, Lovly CM. Mechanisms of receptor tyrosine kinase activation in cancer. *Mol Cancer.* 2018;17(1):58.
42. Ongusaha PP, Kim JI, Fang L, et al. p53 induction and activation of DDR1 kinase counteract p53-mediated apoptosis and influence p53 regulation through a positive feedback loop. *EMBO J.* 2003;22(6):1289–1301.
43. Ruiz PA, Jarai G. Collagen I induces discoidin domain receptor (DDR) 1 expression through DDR2 and a JAK2-ERK1/2-mediated mechanism in primary human lung fibroblasts. *J Biol Chem.* 2011;286(15):12912–12923.
44. Davis ME. Ocular toxicity of tyrosine kinase inhibitors. *Oncol Nurs Forum.* 2016;43(2):235–243.
45. Hammer-Jespersen K, Munch-Petersen A. Phosphodeoxyribomutase from *Escherichia Coli*. Purification and some properties. *Eur J Biochem.* 1970;17(3):397–407.
46. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J.* 2008;22(3):659–661.

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