Changes in clinical outcomes and inflammatory tear cytokine levels in patients with moderate and severe meibomian gland dysfunction treated with various medicines and eyelid scrubs with warm compresses

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Directed by Professor Tae-im Kim

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ABSTRACT

Changes in clinical outcomes and inflammatory tear cytokine levels in patients with moderate and severe meibomian gland dysfunction treated with various medicines and eyelid scrubs with warm compresses

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Purpose: To assess clinical outcomes and tear cytokine levels in moderate and severe meibomian gland dysfunction (MGD) after 2 months of treatment with oral minocycline, topical artificial tears, topical loteprednol etabonate, and eyelid scrubs with warm compresses.

Methods: Patients with stage 3 or 4 MGD were divided into four groups (Group I, 50 mg minocycline two times a day and 0.1% sodium hyaluronate four times a day; Group II, 0.1% sodium hyaluronate four times a day; Group III, 0.5% loteprednol etabonate ophthalmic suspension four times a day following eyelid scrubs with warm compresses two times a day; and Group IV, eyelid scrubs with warm compresses two times a day). We evaluated tear film break-up time (TBUT), corneal and conjunctival fluorescein staining, biomicroscopic examination of lid margins and meibomian glands, Ocular Surface Disease Index (OSDI), and tear cytokine levels before treatment and at 1 and 2 months after treatment.

Results: In linear mixed model (LMM), regarding the interaction effect between groups and time courses, there were statistically significant

differences in the measurement of TBUT, corneal and conjunctival fluorescein staining, conjunctival fluorescein staining, OSDI scores, and meibum quality (P = 0.002 for TBUT, P = 0.038 for corneal fluorescein staining, P < 0.001 for conjunctival fluorescein staining, P = 0.001 for DEWS staining score, P = 0.002 for Oxford staining score, P = 0.030 for OSDI scores, and P < 0.001 for meibum quality). Using a generalized estimating equations model, we found significant differences in lid margin abnormality, meibomian gland expressibility, ocular irritation symptom scores, and MGD stage (all P < 0.001). LLM with adjustment for baseline cytokine concentrations revealed statistically significant differences in the measurement of interleukin (IL)-8 and monocyte chemotactic protein-1 (MCP-1) during the treatment period (P = 0.008 for IL-8 and P = 0.001 for MCP-1). Improvement in overall MGD stage and tear cytokine levels were remarkable in Groups I and III.

Conclusions: Oral minocycline and topical loteprednol etabonate may provide better clinical benefits and anti-inflammatory effects than non-preserved artificial tears or simple eyelid scrubs with warm compresses alone.

Key words: meibomian gland dysfunction; oral minocycline; topical loteprednol etabonate; tear inflammatory cytokines; meibomian gland dysfunction stage

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I. INTRODUCTION

Blepharitis is a common ocular surface disorder with a complex etiology which affects approximately 39-50% of the adult population.[1-3] It may be associated with several systemic diseases, particularly rosacea and seborrheic dermatitis, and is related to other ocular conditions like dry eye, chalazion, conjunctivitis, and keratitis.[4-6] Common symptoms are burning sensation, irritation, tearing, photophobia, blurred vision, and red eyes.[7,8] Objective signs, which often accompany these symptoms, include eyelid crusting and/or loss of eyelashes, eyelid margin redness, conjunctival redness, hyperkeratinization of eyelid, telangiectasia of the lid margin, increased discharge from the meibomian gland, and irregular thickening of the dirty lipid layer.[8]

Meibomian gland dysfunction (MGD), a particular type of posterior blepharitis, is a prevalent condition and one of the major causes of dry eye syndrome.[2,9] MGD is usually secondary to structural changes or dysfunction of the meibomian glands.[10] MGD is a chronic, diffuse abnormality of the meibomian glands and is commonly characterized by terminal duct obstruction and changes in glandular secretions.[10] Modified and deficient meibum lipids result in tear instability, evaporative dry eye, and eyelid inflammation, which are all common

detectable signs of MGD.[11,12]

In one study evaluating the possible association of tear proteins with the severity of MGD in dry eye, increasing levels of distinct tear proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B), were correlated with MGD severity.[13] Many investigators have reported that the chronic inflammatory status in patients with MGD is associated with high concentrations of tear cytokines.[14-17] One study compared inflammatory tear cytokine levels between MGD patients and normal controls and found that concentrations of interleukin (IL)-6 and pro-matrix metalloproteinase (MMP)-9 were found to be significantly higher in MGD patients.[14] Moreover, a strong interaction between MMP and inflammatory cytokines has been reported, describing how each activated the other type of mediator from its respective inactive precursor.[18] Higher concentrations of IL-6, IL-8, IL-12, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were reported in dysfunctional tear syndrome with MGD.[15] MMP-9 was shown to be significantly elevated and correlated with IL-1α in MGD associated with ocular rosacea.[19] Both IL-1β and IL-17 levels were found to be elevated in MGD patients' tears.[16,17]

Eyelid management, including warm compresses and lid scrubs, is a conservative and traditional treatment modality for MGD[20,21] and thought to improve meibomian gland function and ocular comfort by melting and releasing the abnormally modified meibum.[1] However, simple eyelid management should be supplemented with additional treatment to relieve obstructed meibomian glands efficiently and modulate subsequent inflammatory processes in moderate and severe cases of MGD.[20,22] Thus, eyelid management needs to be supported by additional treatment to achieve a satisfactory and quick response.

Topical azithromycin, a macrolide antibiotic with presumed anti-inflammatory effects, has been reported to be effective for treating MGD.[23] In a recent study, azithromycin was shown to suppress inflammatory cytokines (TNF-α, IL-1β), chemokines (IL-8, RANTES), and MMP (MMP-1,3 and 9) by blocking nuclear

factor-kappa B activation in human corneal epithelial cells.[24] Both topical cyclosporine and diquafosol have also shown promising results in treating MGD.[25,26] Systemic tetracycline, doxycycline, and minocycline shown to be effective in treating moderate to severe MGD through their anti-inflammatory, anti-metalloproteinase, and anti-apoptotic properties.[20,27] In one study, two months of oral minocycline treatment was shown to not only improve clinical signs and symptoms, but also decrease IL-6, IL-1β, IL-17α, TNF-α, and IL-12p70 in patients with moderate and severe MGD.[27] Because minocycline is well tolerated with excellent bioavailability, absorption, a prolonged half-life, and a highly lipophilic nature, it could be a potential therapeutic option in MGD.[28] Loteprednol etabonate, a novel C_{20} ester-based corticosteroid, was retrometabolically designed to provide potent anti-inflammatory effects with a decreased impact on intraocular pressure (IOP).[29] It has been shown that after exerting its therapeutic effects at the site of action, loteprednol etabonate is rapidly converted to inactive metabolites, thereby resulting in fewer adverse effects.[29] Several clinical studies evaluating the efficacy of 0.5% loteprednol etabonate ophthalmic suspension in patients with acute anterior uveitis, giant papillary conjunctivitis, seasonal allergic conjunctivitis, postoperative inflammation, and ocular pain, have shown the efficacy of this medication.[30-32] In addition, topical loteprednol etabonate treatment is associated with a relatively lower risk of clinically significant increases in IOP (10 mmHg or higher).[33,34] Topical loteprednol etabonate has been shown to provide satisfactory anti-inflammatory effects and clinical benefits through the regulation of inflammatory tear cytokines IL-6, IL-8, and IL-1β, without serious adverse events.[35]

To our knowledge, there has been no comparison study on the clinical outcomes and tear cytokine levels in moderate and severe MGD patients treated with various treatment modalities including oral minocycline, topical loteprednol etabonate, topical artificial tears, and eyelid scrubs with warm

compresses. Therefore, in the present study, we evaluated and compared the effect of each treatment modality on clinical outcomes and changes of IL-6, IL-7, IL-8, IL-1β, IL-17α, IL-12p70, monocyte chemotactic protein-1 (MCP-1), TNF-α, and IFN-γ levels in moderate and severe MGD.

II. MATERIALS AND METHODS

1. SUBJECTS

This controlled comparison study was conducted according to the Declaration of Helsinki and Good Clinical Practices. Informed consent was obtained from all patients after explanation of the purpose and possible consequences of the study. Inclusion criteria included patients with moderate and severe MGD.[22] MGD was diagnosed by evidence of lid margin or tarsal conjunctival erythema, bulbar conjunctival hyperemia, telangiectasia, thickening, irregularity of the eyelid margins, and/or meibomian gland orifice inclusions. The stage of MGD was assessed by evaluating conjunctival inflammation, clinical symptoms, corneal and conjunctival fluorescein staining, as well as clinical signs including lid margin abnormality, expressibility, and altered secretions.[22] Moderate MGD was defined as moderate symptoms of ocular discomfort, itching or photophobia; moderate MGD clinical signs (plugging, vascularity, moderately altered secretions of grade $\geq$8 to <13, expressibility 2); or mild to moderate conjunctival and peripheral corneal staining (Dry Eye Workshop [DEWS] grade 8–23, Oxford grade 4–10).[22] Severe MGD was defined as marked symptoms of ocular discomfort, itching or photophobia; severe MGD clinical signs (dropout, displacement, severely altered secretions of grade $\geq$ 13, expressibility 3); increased conjunctival and corneal staining (DEWS grade 24–33, Oxford grade 11–15); or increased signs of inflammation (moderate conjunctival hyperemia, phlyctenules).[22] Exclusion criteria included a history of previous ocular or intraocular surgery, glaucoma or ocular hypertension, ocular infection, non-dry eye ocular inflammation, ocular allergy, autoimmune disease, history of intolerance or hypersensitivity to any component of the study medications, use of contact lenses during the study period, presence of current punctal occlusion, pregnancy, children, and lactating women. After a wash-out period of 2 weeks for patients using any other topical or systemic medication, enrolled patients were allocated into one of four groups; Group I, 50 mg minocycline (Minocin,

SK chemical, Seoul, Korea) two times a day and 0.1% sodium hyaluronate (Kynex, Alcon Laboratory, Seoul, Korea) four times a day; Group II, 0.1% sodium hyaluronate four times a day; Group III, 0.5% loteprednol etabonate ophthalmic suspension (Lotemax; Bausch and Lomb Inc., Rochester, NY, USA) four times a day following eyelid scrubs with warm compresses two times a day; and Group IV, eyelid scrubs with warm compresses two times a day. The eye with the higher stage of MGD was chosen to be the study eye. If the MGD stages for each eye were equal, the right eye was enrolled as the test eye. To minimize the extent to which one test influenced the results of the tests that followed, each test was routinely performed in the following order: tear collection, biomicroscopic examination of tear break up time (TBUT), corneal and conjunctival fluorescein staining, examination of lid margins and meibomian glands, and the Ocular Surface Disease Index (OSDI) questionnaire. At least 10 min were allowed between tests. Patients received instructions on how to perform standard eyelid management via face-to-face education at every follow-up time, and were instructed not to wipe or scrub their eyelid margins on the day of tear sampling.[20]

2. TEAR COLLECTION AND MULTIPLEX BEAD ANALYSIS

For tear cytokine analysis, 30 μL of phosphate-buffered saline was injected into the inferior conjunctival sac using a micropipette.[27] Approximately 20 μL of tear fluid in buffer was collected with a micropipette. In order to minimize irritation of the ocular surface or lid margin, unstimulated tear fluid was collected from the marginal tear strip of the lower lid near the lateral canthus. Anesthetic drops were not administered. Tear samples were immediately transferred to 0.5 mL Eppendorf tubes (Eppendorf, Fremont, CA, USA) and placed on dry ice. The tubes were frozen at -70℃ until they were used for immunoassay. Cytokines were measured using the BD Cytometric Bead Array (BD Bioscience, San Jose, CA, USA). The cytokines analyzed were IL-6, IL-7, IL-8, IL-1β, IL-17α,

IL-12p70, MCP-1, TNF-α, and IFN-γ. Cytokine level measurements were performed essentially as previously described.[36] Briefly, 20 μL of tear fluid were thawed and added to a 50 μL mixture containing capture antibody-bead reagent and 50 μL detector antibody-phycoerythrin reagent. The mixture was subsequently incubated for 3 hours at room temperature and washed to remove unbound detector antibody-phycoerythrin reagent before flow cytometry. Data were obtained and analyzed using BD Cytometric Bead Array software that calculated the cytokine concentration based on standard curves and a four parameter logistic curve-fitting model. Flow cytometry was performed using the BD LSRII system (BD Bioscience).

3. OCULAR SURFACE EVALUATIONS

A. TEAR BREAK UP TIME

To measure TBUT, a drop of non-preserved saline solution was added to a fluorescein strip (Haag-Streit, Koeniz, Switzerland), which was applied to the inferior palpebral conjunctiva. Patients were instructed to blink 3 or 4 times for a few seconds to ensure adequate mixing of the dye, then the eye was examined using a slit lamp with maximum cobalt blue light. Patients were asked to open their eye wide and look straight ahead. Using a stop watch, the physician measured the time it took for a single black dot or line to appear on the cornea. The test was performed 3 times and the average time was calculated.

B. CORNEAL AND CONJUNCTIVAL FLUORESCEIN STAINING SCORES

Staining scores were obtained by adding the following scores of the exposed cornea and conjunctiva: Oxford staining score (range: 1-15) and DEWS staining score (range: 0-33).

C. MEIBOMIAN GLAND DYSFUNCTION STAGING

Microscopic examination of the meibomian glands was performed last because this process could affect the results of the other tests. The operator allocated a score from 0-4 for the presence or absence of lid margin abnormalities, including lid margin irregularity, plugging of the meibomian orifices, lid margin vascular engorgement, and anterior or posterior replacement of mucocutaneous junction.[37] The degree of expressibility using firm digital pressure applied over five lower lid glands was based on the following: grade 0, all five glands expressible; grade 1, three to four glands expressible; grade 2, one to two glands expressible; grade 3, 0 glands expressible.[38] The degree of meibum quality using firm digital pressure applied over eight lower lid glands was based on the following: grade 0, clear; grade 1, cloudy; grade 2, cloudy with granular debris; grade 3, thick, toothpaste-like consistency. Each of the eight glands of the lower eyelid was graded on a scale from 0 to 3. The scores of the eight glands were added together to obtain a total score (maximum score for each eye was 24). Staining scores were obtained by adding the scores of the exposed cornea and conjunctiva (Oxford staining score range, 1-15; DEWS staining score range, 0-33). Patients were asked to rate subjective symptoms (ocular discomfort, itching, and photophobia with limitations of activities) on a scale of 0 (no symptoms) to 3 (severe symptoms). The stage of MGD was assessed using the clinical parameters and the symptom questionnaire.

4. SUBJECTIVE SYMPTOMS

All patients completed the validated 12 item OSDI questionnaire that were assessed on a scale from 0 to 100, with higher scores representing greater disability.[39] The total OSDI score was calculated using the following formula: OSDI score = (sum of scores for all questions answered × 100)/(total number of questions answered × 4).[39]

5. ASSESSMENT OF SAFETY

Safety was assessed by monitoring any adverse events during the course of the study. Patients were also educated to report any unfavorable symptoms or signs, such as itching, irritation, and hyperemia or gastrointestinal discomfort.

6. STATISTICAL ANALYSIS

A linear mixed model with post-hoc analysis was used to evaluate possible differences between the four groups and two or three time courses in the measurement of clinical outcomes and cytokine levels, with the unstructured covariance matrix or compound symmetry covariance matrix. A generalized estimating equations model was used for the evaluation of non-continuous scale values, such as lid margin abnormality, expressibility, ocular irritation symptom score, and MGD stage. Statistical analysis was performed using SAS software (version 9.2; SAS Institute, Inc., Cary, NC, USA). Differences were considered statistically significant when the *P* values were less than 0.05.

III. RESULTS

Patients' characteristics are summarized in Table 1.

Table 1. Characteristics of patients treated with oral minocycline, topical artificial tears, topical loteprednol etabonate, and eyelid scrubs with warm compresses

	Group I[1]	Group II[2]	Group III[3]	Group IV[4]	*P*
Patients (eye)	21 (21)	19 (19)	30 (30)	30 (30)	
Gender					0.395
Female	13	16	19	20	
Male	8	3	11	10	
Laterality					0.219
Right	15	14	15	16	
Left	6	5	15	14	
Age (years)					0.505

Mean (SD)	63.2 (9.3)	63.8 (13.5)	66.8 (10.1)	67.1 (11.7)
Range	46-78	43-80	46-81	44-81

No significant difference among groups was detected
SD, standard deviation
[1]50 mg minocycline two times a day and 0.1% sodium hyaluronate four times a day
[2]0.1% sodium hyaluronate four times a day
[3]0.5% loteprednol etabonate ophthalmic suspension four times a day following eyelid scrubs with warm compresses
[4]eyelid scrubs with warm compresses

No significant differences in any parameters were found among the groups before treatment. Figure 1 shows the results of BUT, corneal fluorescein staining, conjunctival fluorescein staining, DEWS, and Oxford staining score before and after treatment.

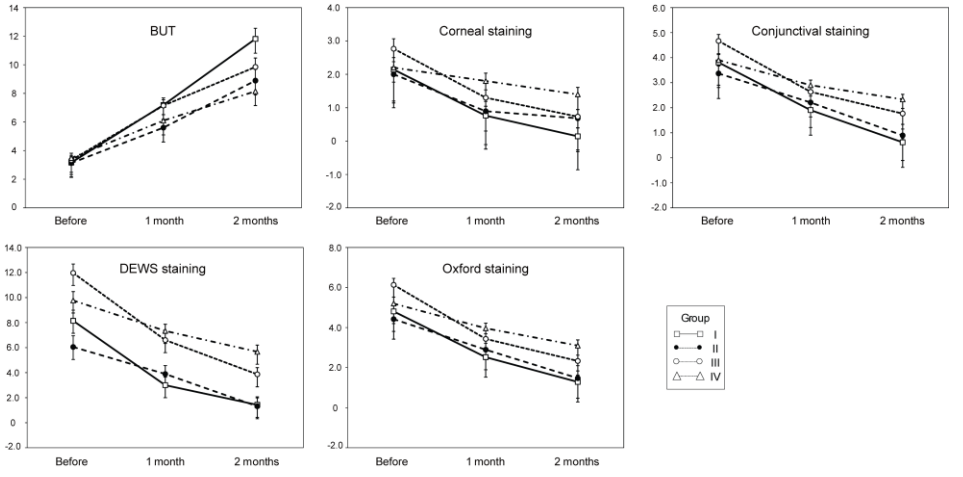

Figure 1. Change of tear break up time and corneal and conjunctival fluorescein staining score during treatment period. Group I, oral minocycline and artificial tears; Group II, artificial tears; Group III, topical loteprednol etabonate and eyelid scrubs with warm compresses; Group IV, eyelid scrubs with warm compresses; BUT, break up time; DEWS, Dry Eye Workshop. Error bars represent standard error for least squares means

In the linear mixed model, regarding the interaction effect between groups and time courses, there were statistically significant differences in the measurement of TBUT, corneal fluorescein staining, conjunctival fluorescein staining, DEWS,

and Oxford staining scores (P = 0.002 for TBUT, P = 0.038 for corneal fluorescein staining, P < 0.001 for conjunctival fluorescein staining, P = 0.001 for DEWS staining score, and P = 0.002 for Oxford staining score; Figure 1 and Table 2).

Table 2. Clinical signs and symptoms before and after treatment with oral minocycline, topical artificial tears, topical steroid, and eyelid scrubs with warm compresses in moderate and severe meibomian gland dysfunction

	Group I[1]			Group II[2]			Group III[3]			Group IV[4]			P[5]
	Baseline	1 M	2 M	Baseline	1 M	2 M	Baseline	1 M	2 M	Baseline	1 M	2 M	
TBUT (sec)	3.17 (0.40)	7.19 (0.49)	11.81 (0.74)	3.13 (0.42)	5.61 (0.52)	8.89 (0.78)	3.33 (0.34)	7.15 (0.41)	9.85 (0.62)	3.48 (0.34)	6.10 (0.41)	8.15 (0.62)	0.002
Staining score													
Cornea	2.14 (0.36)	0.76 (0.28)	0.14 (0.25)	2.00 (0.38)	0.89 (0.30)	0.68 (0.26)	2.77 (0.30)	1.30 (0.23)	0.73 (0.21)	2.20 (0.30)	1.80 (0.23)	1.40 (0.21)	0.038
Conjunctiva	3.81 (0.31)	1.90 (0.24)	0.62 (0.24)	3.37 (0.33)	2.21 (0.26)	0.89 (0.25)	4.67 (0.26)	2.63 (0.20)	1.77 (0.20)	3.90 (0.26)	2.90 (0.20)	2.33 (0.20)	<0.001
DEWS	8.14 (0.85)	3.00 (0.63)	1.43 (0.64)	6.05 (0.89)	3.89 (0.67)	1.32 (0.67)	11.97 (0.71)	6.60 (0.53)	3.87 (0.54)	9.77 (0.71)	7.33 (0.53)	5.67 (0.54)	0.001
Oxford	4.81 (0.38)	2.52 (0.31)	1.29 (0.34)	4.42 (0.40)	2.89 (0.32)	1.47 (0.36)	6.13 (0.32)	3.43 (0.26)	2.33 (0.29)	5.20 (0.32)	3.97 (0.26)	3.10 (0.29)	0.002
Eyelid													
Meibum quality	12.90 (0.57)	8.86 (0.58)	4.62 (0.62)	11.42 (0.60)	9.26 (0.61)	8.42 (0.65)	17.73 (0.48)	10.73 (0.48)	5.07 (0.52)	17.17 (0.48)	13.20 (0.48)	8.83 (0.52)	<0.001
Subjective score													
OSDI	23.38 (1.93)	9.10 (1.60)	4.33 (1.01)	20.68 (2.03)	12.68 (1.68)	6.11 (1.06)	22.67 (1.61)	11.73 (1.34)	3.33 (0.84)	26.63 (1.61)	13.73 (1.34)	6.50 (0.84)	0.030

Results are presented as least square mean (standard error)
M, month; TBUT, tear break up time; DEWS, Dry Eye Workshop; OSDI, ocular surface disease index
[1]50 mg minocycline two times a day and 0.1% sodium hyaluronate four times a day
[2]0.1% sodium hyaluronate four times a day
[3]0.5% loteprednol etabonate ophthalmic suspension four times a day following eyelid

scrubs with warm compresses
[4]eyelid scrubs with warm compresses
[5]linear mixed model with post hoc analysis considering the interaction effect between the 4 groups and the 3 time courses

The mean TBUT at 2 months after treatment showed significant improvement in all groups when compared with baseline ($P < 0.001$). There were significant differences in the mean BUT between the groups at 2 months after treatment ($P = 0.049$ for Group I vs Group II and $P = 0.002$ for Group I vs Group IV). Changes in TBUT in Group I were significantly larger than those in Group IV when comparing before treatment with 2 months after treatment ($P = 0.001$). Corneal fluorescein staining scores at 2 months after treatment showed significant improvement when compared with baseline in all groups ($P < 0.001$ for Groups I, II and III, and $P = 0.009$ for Group IV). At 2 months after treatment, Group I showed better results than Group IV with regards to corneal fluorescein staining scores ($P = 0.001$). Changes in corneal fluorescein staining scores in Group III were significantly larger than those in Group IV when comparing before treatment with 2 month after treatment ($P = 0.026$). Conjunctival fluorescein staining scores at 2 months after treatment showed significant improvement when compared with baseline in all groups ($P < 0.001$). Changes in conjunctival fluorescein staining scores in Group IV were significantly smaller than those in Groups I and III when comparing before treatment with 2 month after treatment ($P < 0.001$). DEWS and Oxford staining scores at 2 months after treatment showed significant improvement when compared with baseline in all groups ($P < 0.001$). Changes in DEWS scores in Group III were significantly larger than those in Groups II and IV when comparing before treatment with 2 month after treatment ($P = 0.023$ and $P = 0.001$). Changes in Oxford scores in Group IV were significantly smaller than those in Groups I and III when comparing before treatment with 2 month after treatment ($P = 0.024$ and $P = 0.001$). Figure 2 shows the results of meibum quality and OSDI score before and after treatment.

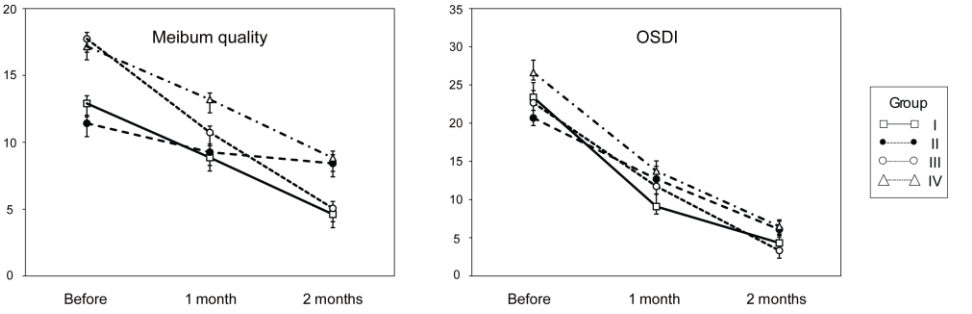

Figure 2. Change of meibum quality and ocular surface disease index scores during treatment period. Group I, oral minocycline and artificial tears; Group II, artificial tears; Group III, topical loteprednol etabonate and eyelid scrubs with warm compresses; Group IV, eyelid scrubs with warm compresses; OSDI, ocular surface disease index. Error bars represent standard error for least squares means

In the linear mixed model, regarding the interaction effect between groups and time courses, there were statistically significant differences in the measurement of meibum quality ($P < 0.001$; Figure 2 and Table 2). Meibum quality at 2 months after treatment showed significant improvement when compared with baseline in all groups ($P < 0.001$). Changes in meibum quality in Group II were significantly smaller than those in Groups I, III and IV when comparing before treatment with 2 month after treatment ($P < 0.001$). Changes in meibum quality in Group III were significantly larger than those in Groups I, II and IV when comparing before treatment with 2 month after treatment ($P < 0.001$). In the linear mixed model, regarding the interaction effect between groups and time courses, there were statistically significant differences in the measurement of OSDI ($P = 0.030$; Figure 2 and Table 2). However, changes in OSDI during the treatment period were comparable among the groups. Figure 3 demonstrates the results of lid margin abnormality, meibomian gland expressibility, ocular irritation symptom scores, and MGD stage before and after treatment.

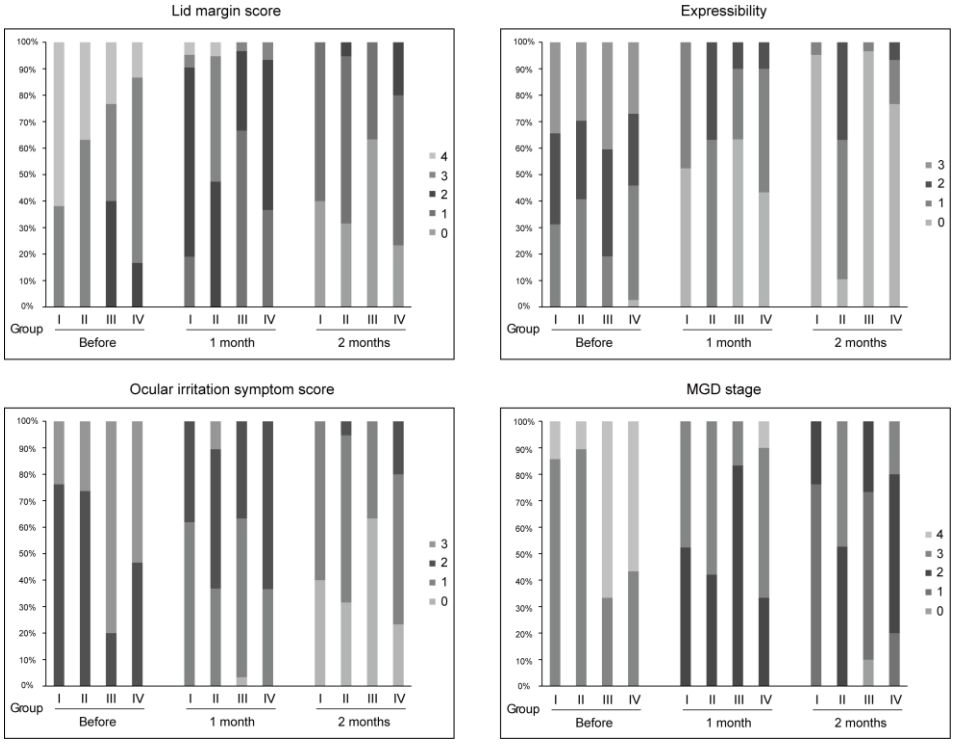

Figure 3. Change of lid margin abnormality, meibomian gland expressibility, ocular irritation symptom scores and meibomian gland dysfunction stage during treatment period. Group I, oral minocycline and artificial tears; Group II, artificial tears; Group III, topical loteprednol etabonate and eyelid scrubs with warm compresses; Group IV, eyelid scrubs with warm compresses; MGD, meibomian gland dysfunction

Using a generalized estimating equations model, there were statistically significant differences in lid margin abnormality and meibomian gland expressibility ($P < 0.001$; Figure 3). Results showed the superior efficacy of oral minocycline for improving lid margin abnormality (odds ratio = 13.19, $P < 0.001$ for Group I vs. II, odds ratio = 6.42, $P = 0.020$ for Group I vs. III, and odds ratio = 20.31, $P < 0.001$ for Group I vs. IV). Results also showed the superior efficacy of oral minocycline for improving meibomian gland expressibility (odds ratio = 65.15, $P < 0.001$ for Group I vs. II, odds ratio = 11.49, $P < 0.001$ for Group I vs. III, and odds ratio = 23.50, $P < 0.001$ for

Group I vs. IV). Regarding meibomian gland expressibility, artificial tears alone demonstrated inferior results over topical loteprednol etabonate following eyelid scrubs with warm compresses (odds ratio = 0.18, P = 0.036). Using a generalized estimating equations model, there were statistically significant differences in ocular irritation symptoms and MGD stage (P < 0.001; Figure 3). Results showed an inferior efficacy of topical artificial tears for improving ocular irritation symptoms when compared with the other groups (odds ratio = 41.31, P < 0.001 for Group I vs. II, odds ratio = 0.10, P = 0.004 for Group II vs. III, and odds ratio = 0.07, P = 0.011 for Group II vs. IV). Regarding MGD stage, we demonstrated an inferior efficacy of topical artificial tears when compared with the other groups (odds ratio = 75.92, P < 0.001 for Group I vs. II, odds ratio = 0.17, P < 0.001 for Group II vs. III, and odds ratio = 0.03, P < 0.011 for Group II vs. IV).

Figure 4 demonstrates a comparison of tear cytokine concentrations before and after treatment without adjustment of baseline cytokine concentrations. In Group I, levels of IL-6, IL-1β and IL-12p70 significantly decreased after 2 months of treatment. In Group III, topical loteprednol etabonate following eyelid scrubs with warm compresses significantly decreased the levels of IL-6, IL-7, IL-8, IL-1β, and MCP-1 after 2 months of treatment. In Group IV, levels of IL-8 significantly decreased after 2 months of treatment.

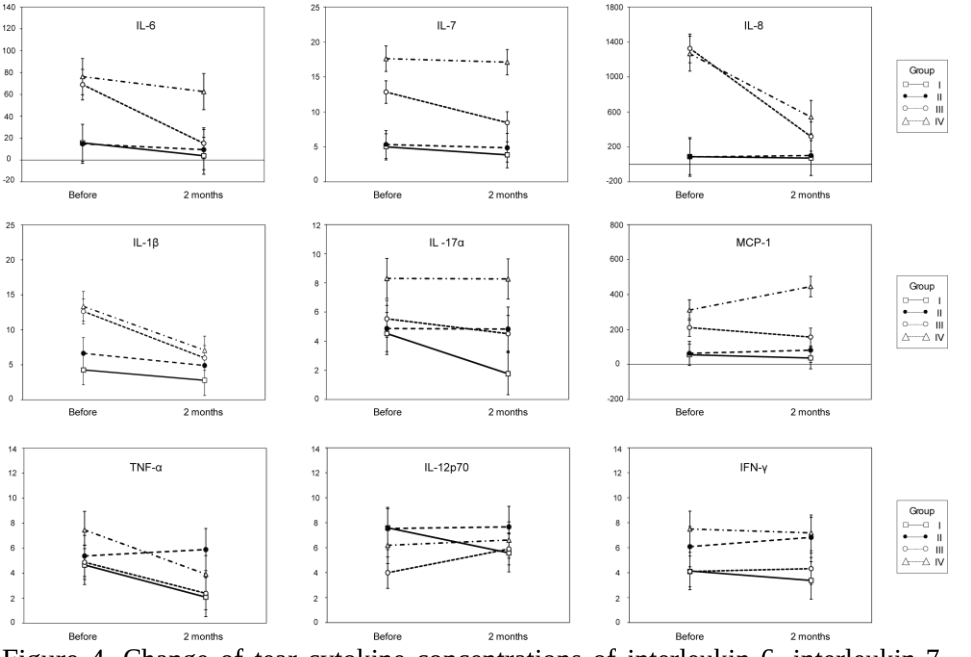

Figure 4. Change of tear cytokine concentrations of interleukin-6, interleukin-7, interleukin-8, interleukin-1β, interleukin-17α, monocyte chemotactic protein-1, tumor necrosis factor-α, interleukin-12p70, and interferon-γ during treatment period. Group I, oral minocycline and artificial tears; Group II, artificial tears; Group III, topical loteprednol etabonate and eyelid scrubs with warm compresses; Group IV, eyelid scrubs with warm compresses; IL, interleukin; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor; IFN, interferon. Error bars represent standard error for least squares means

Using a linear mixed model with adjustment for baseline cytokine concentrations, there were statistically significant differences in the measurement of IL-8 and MCP-1 (P = 0.008 for IL-8 and P = 0.001 for MCP-1; Figure 5).

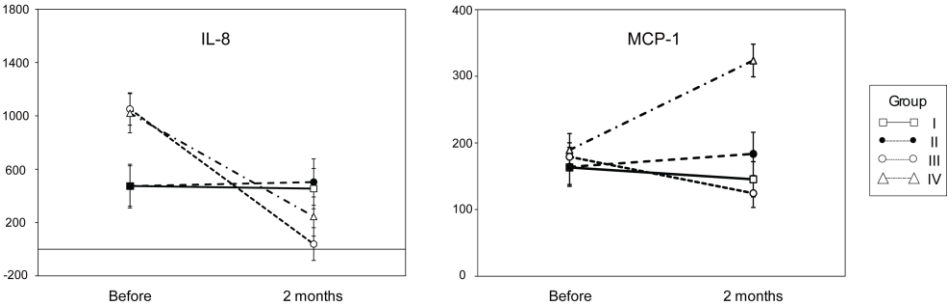

Figure 5. Change of tear cytokine concentrations of interleukin-8 and monocyte chemotactic protein-1 during treatment period using linear mixed model with adjustment for baseline cytokine concentrations. Group I, oral minocycline and artificial tears; Group II, artificial tears; Group III, topical loteprednol etabonate and eyelid scrubs with warm compresses; Group IV, eyelid scrubs with warm compresses; IL, interleukin; MCP, monocyte chemotactic protein. Error bars represent standard error for least squares means

Changes in concentrations of IL-8 in Group III were significantly larger than those in Group I when comparing before treatment with 2 months after treatment (P = 0.035; Figure 5). Changes in concentrations of IL-8 in Group III were larger than those in Group II when comparing before treatment with 2 months after treatment (P = 0.039; Figure 5). Regarding MCP-1, oral minocycline effectively decreased the levels of MCP-1 when compared with simple eyelid scrubs with warm compresses alone (P = 0.025 for Group I vs. IV; Figure 5). Topical loteprednol etabonate following eyelid scrubs with warm compresses effectively decreased the levels of MCP-1 when compared with simple eyelid scrubs with warm compresses alone (P = 0.001 for Group III vs. IV; Figure 5).

After 1 month of treatment with oral minocycline, gastrointestinal side effects were reported by two patients. These two patients stopped medication, and the symptoms abated. No other unfavorable symptoms or signs were reported. These two cases were not included in the analysis.

IV. DISCUSSION

In the present study, we evaluated and compared the efficacy of oral

minocycline, topical loteprednol etabonate, topical artificial tears, and eyelid scrubs with warm compresses by evaluating the clinical signs and symptoms and changes of IL-6, IL-7, IL-8, IL-1β, IL-17α, IL-12p70, MCP-1, TNF-α, and IFN-γ levels in patients with moderate and severe MGD.

Overall, keratoconjunctival fluorescein staining scores, such as DEWS and Oxford staining scores, demonstrated that oral minocycline was more effective than simple eyelid scrubs with warm compresses. Topical loteprednol etabonate had additional positive effects when combined with eyelid scrubs with warm compresses regarding with results of corneal and conjunctival fluorescein staining scores. Regarding tear film stability, oral minocycline showed more effective ocular surface stabilization when compared with simple eyelid scrubs with warm compresses. Although topical artificial tears showed comparable results regarding BUT and fluorescein staining scores, this treatment modality did not significantly affect lid margin abnormality, meibomian gland expressiblity and meibum quality. Regarding lid margin abnormality and meibomian gland expressibility, oral minocycline effectively improved lid margin status, which consequently increased meibomian gland expressibility. Topical loteprednol etabonate led to significant reductions in lid margin abnormality, meibum quality, and expressibility, which are all important indicators of eyelid inflammation. Furthermore, topical loteprednol etabonate following eyelid scrubs with warm compresses effectively improved meibum quality when compared with oral minocycline, topical artificial tears, or eyelid scrubs with warm compresses alone. Improvements in the quality of the meibum and reduction in meibomian gland orifice plugging may help stabilize the ocular surface by improving the quality and quantity of the tear film lipid layer. Observed restoration of ocular surface integrity and improvements in eyelid inflammation may subsequently lead to a reduction in the OSDI and ocular irritation symptom scores. The reduction of MGD stage encompassing a variety of clinical signs and symptoms was most noteworthy in MGD patients

treated with oral minocycline and topical loteprednol etabonate.

The pathogenesis of MGD is still unclear and probably multifactorial.[1] Alterations to the anatomy of the meibomian glands and their secretions contribute to the increased evaporation of tear film, increased tear osmolarity, and increased lid inflammation which ultimately damages the ocular surface.[40-43] Increasingly, it is proposed that inflammation may be associated with the development of MGD. Supporting evidence includes reports of increased tear concentrations of IL-6, IL-8, IL-12, IFN-γ, TNF-α, IL-1β, IL-17, and MMP-9 in cases of dysfunctional tear syndrome with MGD.[14-17] Alteration of cytokine levels has been associated with epithelial keratinization, relative hypothesia, altered mucin secretion, and corneal neovascularization, resulting in tear film instability and non-wettable ocular surfaces, which eventually contribute to a disrupted integrity of the ocular surface.[44-47] Subsequent cytokine secretion by the damaged ocular surface epithelial cells can then perpetuate ocular surface inflammation.[48]

Regarding inflammatory tear cytokine levels before and after treatment, oral minocycline significantly decreased the levels of IL-6, IL-1β, and IL-12p70 after 2 months of treatment. Pleiotropic proinflammatory cytokine IL-6, which is associated with the severity of dry eye syndrome, is induced by desiccating stress in corneal epithelial cells and can induce the production of MMPs.[49,50] IL-1β is a potent inducer of IL-6, IL-8, and TNF-α, and can stimulate the production of MMPs.[16] In some disease states, increased IL-1 levels in tears play a key role in the damage and keratinization of the ocular surface.[16,51] Although topical artificial tears showed comparable results regarding BUT and fluorescein staining scores, they did not affect eyelid margin and inflammatory tear cytokine levels. Thus, we assume that oral minocycline effectively improved lid margin status, meibomian gland expressibility, and various ocular surface parameters by decreasing the cytokine levels, which consequently resulted in a definite decrease in MGD stage.

Systemic tetracycline, doxycycline, and minocycline have also been reported to be effective in treating MGD.[52-56] These medications may be helpful for blocking the self-propagating characteristics of MGD through their anti-inflammatory, anti-metalloproteinase, and anti-apoptotic properties. Furthermore, they counteract free fatty acid accumulation which can be toxic to ocular tissue and has an adverse effect on tear film.[14,53,55,57-59] Oral minocycline treatment has been shown to decrease eyelid bacterial flora effectively in patients with acne rosacea or blepharitis.[56] Minocycline suppressed the production of neutrophil chemotactic factors and reactive oxygen species, leading to decreased inflammation.[60] Minocycline has been reported to attenuate TNF-α, IL-1β, IL-8, and IL-6 production.[61-64] It increased the levels of IL-12p40, which at high levels may antagonize pro-inflammatory IL-12 receptors, thereby decreasing the effects of mature IL-12p70.[65,66] Minocycline also has suppressive effects on inflammatory cells, such as T lymphocytes and monocytes, resulting in an inhibitory effect on TNF-α, MMP-9 and IFN-γ production.[67,68] It could be postulated that minocycline may interfere with the activation of T cells in the ocular surface, causing the suppression of pro-inflammatory cytokines which may induce a chain reaction with the infiltrating macrophages and B cells. In addition, minocycline significantly decreased diglycerides and free fatty acid.[55]

In this study, topical loteprednol etabonate following eyelid scrubs with warm compresses significantly decreased the levels of IL-6, IL-7, IL-8, IL-1β, and MCP-1 after 2 months of treatment. Furthermore, it effectively decreased the levels of IL-8 when compared with oral minocycline or topical artificial tears. And, it effectively decreased the levels of MCP-1 when compared with simple eyelid scrubs with warm compresses alone. Considering that topical loteprednol etabonate led to significant improvements in eyelid inflammation and ocular surface parameters, significantly decreased levels of tear cytokines after topical loteprednol etabonate following eyelid scrubs with warm compresses may have important anti-inflammatory effects. Although there is no published report

describing the association between MCP-1 and MGD, MCP-1 is known to be a major pro-inflammatory cytokine and has been linked with the development of T helper cell (Th)1 and Th2.[69,70] Additionally, both IL-8 and MCP-1, which are produced by human corneal tissues in response to inflammatory stimuli, are essential to leukocyte accumulation and activation at sites of inflammation.[69,71] Therefore, it can be assumed that topical loteprednol etabonate not only effectively controlled ocular surface and eyelid inflammation by decreasing tear cytokine levels, but also provided clinical benefits in treating moderate and severe MGD. Consequently, overall MGD stage reduction was definite in MGD patients treated with topical loteprednol etabonate following eyelid scrubs with warm compresses.

Corticosteroids have been reported to decrease the production of IL-6, IL-8, MCP-3, and RANTES produced by the corneal epithelium, and the expression of IL-1α, IL-1β, TNF-α and MMP-9 in the corneal epithelium of experimental dry eye model.[72-74] However, increased IOP and cataract formation are associated with corticosteroid use. In an effort to decrease these adverse effects, loteprednol etabonate was developed based on a retrometabolic drug design and is thought to provide similar therapeutic potency while minimizing any adverse drug reactions.[29] Due to its high lipophilicity, loteprednol etabonate could enhance penetration through biological membranes, thereby increasing its efficacy.[75] Considering the lipid profiles of MGD, it is possible that loteprednol etabonate could penetrate the abnormally modified meibum in the diseased meibomian gland and thereby exert anti-inflammatory effects, not only in the conjunctiva and cornea, but also in the meibomian gland. This supposition is consistent with our findings that loteprednol etabonate improved the ocular surface integrity and decreased eyelid inflammation.

Based on the results of our study, determination of MGD stage can be a good option for planning treatment options and evaluating the efficacy of treatment. We propose that MGD stage evaluation is a valuable tool for investigating the

efficacy of various treatment modalities. Additionally, based on the relationship between inflammatory mediators and ocular surface inflammation or damage, inflammatory cytokines could be targeted to control ocular surface damage in MGD. Moreover, a longitudinal evaluation of changes in tear cytokine levels before and after treatment can be used as objective criteria for diagnosing MGD grade, and analyzing the efficacy of treatment.

The present study's limitations were the small number of patients and the relatively short follow up time of 2 months. Our study was conducted as a randomized clinical trial. However, it was impossible to mask the patients of the treatment groups completely and properly because there was no placebo group. Despite these limitations, we believe our findings may contribute to further investigations evaluating the level of cytokine immunoreactivity or mRNA in ocular surface under the influence of anti-inflammatory treatment in MGD. A larger sample size and longer follow up period would permit a more thorough comparison between treatment modalities. We analyzed a large number of multiple comparisons to detect possible differences. However, this kind of multiplicity may yield a statistical significance by chance. To prevent this problem, we performed a linear mixed model analysis and measured the interaction effect between groups and time courses in the measurement of multiple clinical outcomes and tear cytokine levels.

V. CONCLUSION

We evaluated the efficacy and safety of oral minocycline, topical artificial tears, topical loteprednol etabonate, and eyelid scrubs with warm compresses for the treatment of moderate and severe MGD. Oral minocycline was effective in treating moderate and severe MGD, demonstrating that oral minocycline can provide anti-inflammatory effects and noteworthy improvements in eyelid inflammation. Topical loteprednol etabonate following eyelid scrubs with warm compresses can provide noteworthy anti-inflammatory effects and clinical

benefits through the regulation of inflammatory tear cytokines. Furthermore, tear cytokine measurement and MGD stage assessment can be an additional novel approach for evaluating the efficacy of anti-inflammatory treatment in MGD patients.

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ABSTRACT (IN KOREAN)

네 가지 방법의 치료 전후 임상증상과 눈물 내 염증성 사이토카인 분석을 통한 중등도 이상 마이봄샘 기능장애의 병태생리 및 치료법 제시

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이 훈

목적: 중등도 이상의 마이봄샘 기능장애에서 경구용 미노싸이클린, 무방부제 인공눈물, loteprednol etabonate 점안제, 안검마사지의 치료효과를 임상 양상 및 눈물 내 사이토카인 농도 변화 측면에서 비교분석 하고자 했다.

대상과 방법: 마이봄샘 기능장애 Stage 3 또는 4인 환자를 네 개의 치료군으로 나누었으며 다음과 같다 (Group I; 50 mg 미노싸이클린 2회 복용 및 0.1% sodium hyaluronate 인공눈물 4회 점안, Group II; 0.1% sodium hyaluronate 인공눈물 4회 점안, Group III; 0.5% loteprednol etabonate 점안제 4회 점안 및 안검마사지 2회, Group IV; 안검마사지 2회). 눈물막파괴시간, 각막표면형광염색점수, 결막표면형광염색점수, DEWS 염색점수, Oxford 염색점수, 안검이상 (lid margin abnormality), 마이봄의 상태 (meibum quality), 마이봄의 배출 (meibomian gland expressibility), 환자의 주관적 증상, 눈물 내 사이토카인 농도를 치료 전, 치료 후 1 개월과 2 개월째에 측정 및 분석했다.

결과: 치료군과 치료시점사이의 교호작용을 고려한 linear mixed model (LMM) 결과, 치료군간 눈물막파괴시간, 각막표면형광염색점수, 결막표면형광염색점수, DEWS 염색점수, Oxford 염색점수, 마이봄의 상태의 호전 정도에 유의한 차이가 있었다 (P = 0.002, 눈물막파괴시간; P = 0.038, 각막표면형광염색점수; P < 0.001, 결막표면형광염색점수; P = 0.001, DEWS 염색점수; P = 0.002, Oxford 염색점수; P < 0.001, 마이봄의 상태). Generalized estimating equations model 에 의하면 치료군간 안검이상, 마이봄의 배출, 환자의 주관적 증상, 마이봄샘 기능장애 Stage의 호전 정도에 유의한 차이가 있었다 (P < 0.001). 치료군과 치료시점사이의 교호작용 및 치료 전 사이토카인의 농도까지 고려한 LMM 결과, 치료군간 interleukin (IL)-8 과 monocyte chemotactic protein-1 (MCP-1) 변화 정도에 유의한 차이가 있었다 (P = 0.008, IL-8; P = 0.001, MCP-1). 마이봄샘 기능장애 Stage 및 눈물 내 사이토카인 농도의 변화는 경구용 미노싸이클린과 loteprednol etabonate 점안제로 치료받은 군에서 두드러졌다.

결론: 경구용 미노싸이클린과 loteprednol etabonate 점안제는 중등도 이상의 마이봄샘 기능장애 치료에 있어 임상 양상 및 눈물 내 사이토카인 농도 변화 측면에서 효과적인 치료 방법이다.

핵심되는 말: 마이봄샘 기능장애, 경구용 미노싸이클린, loteprednol etabonate 점안제, 눈물 내 염증 사이토카인, 마이봄샘 기능장애 Stage

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