The Comparison of Efficacies of Topical Corticosteroids and Nonsteroidal Anti-inflammatory Drops on Dry Eye Patients: A Clinical and Immunocytochemical Study

AVNI MURAT AVUNDUK, MD, MUSTAFA CIHAT AVUNDUK, MD, EMILY D. VARNELL, BS, AND HERBERT E. KAUFMAN, MD

• PURPOSE: To investigate whether conjunctival inflammation represents a primary event in the pathogenesis of keratoconjunctivitis sicca or whether it is a secondary inflammatory reaction caused by enhanced mechanical irritation as a result of surface dryness and whether anti-inflammatory drops (corticosteroids and nonsteroidal anti-inflammatory) have therapeutic effects and are similar.

• DESIGN: Single-masked, randomized, prospective clinical trial.

• METHODS: Thirty-two keratoconjuctivitis patients with or without Sjögren syndrome were included in the study. The patients were randomized to three groups. Group 1 patients received a topical artificial tear substitute (ATS); group 2 received ATS plus nonsteroidal anti-inflammatory drops (NSAID); and group 3 received ATS plus topical corticosteroidal drops. The eye symptom severity scores, Schirmer test values, rose bengal and fluorescein staining scores were evaluated before treatment and 15 and 30 days after start of treatment. Impression cytology specimens were stained using immunohistochemical methods to detect the percentages of human leukocyte antigen II (HLA-DR) positive, Apo 2.7 positive, and periodic acid–Schiff positive cells. Statistical analyses were performed within and between groups.

• RESULTS: Group 3 patients had significantly lower symptom severity scores, fluorescein and rose bengal staining, and HLA-DR positive cells on days 15 and 30 compared with patients in other groups. They also had a significantly higher number of periodic acid–Schiff positive (goblet) cells in their impression cytology specimens on days 15 and 30 compared with the other patients. On day 30, group 3 patients had significant differences compared with their baseline measurements in terms of above-mentioned parameters. However, we did not detect a significant effect of any treatment schedule on the Shirmer test value and the numbers of Apo 2.7 cells in impression cytology specimens.

• CONCLUSIONS: Topical corticosteroids had a clearly beneficial effect both on the subjective and objective clinical parameters of moderate-to-severe dry eye patients. These effects were associated with the reduction of inflammation markers of conjunctival epithelial cells. (Am J Ophthalmol 2003;136:593–602. © 2003 by Elsevier Inc. All rights reserved.)

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From the Louisiana State University, School of Medicine, LSU Eye Center, New Orleans, Louisiana (A.M.A., E.D.V., H.E.K.); and Selçuk University, School of Medicine, Department of Pathology, Konya, Turkey (M.C.A.).

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Inquiries to Avni Murat Avunduk, MD, KTU Lojmanlari No: 31/17 61080, Trabzon, Turkey; fax: (+90) 462-3252270; e-mail: avunduk@ttnet.net.tr

ALTHOUGH THE IMMUNOPATHOLOGIC ANALYSIS OF the lacrimal gland has received considerable attention, less work has been done on pathologic changes occurring in the ocular surface of patients with keratoconjunctivitis sicca (KCS). However, a strong expression of human leukocyte antigen II (HLA-DR) antigens has been found in conjunctival epithelial cells of dry eye patients.[1] A strong relationship also has been proposed between inflammatory pathways and apoptosis, which directly affects epithelial turnover.[2] Overexpression of apoptotic markers was shown in impression cytology specimens from patients with KCS.[3,4] The above findings support the immunopathogenesis of KCS. However, it is not clear that this inflammatory reaction represents a primary phenomenon. Conversely, it may result from chronic surface dryness and an increased friction between

palpebral and bulbar conjunctiva because of tear deficiency.

Evidence of inflammatory processes in the pathogenesis of KCS led to the development of cyclosporine A (CsA) as a first attempt to treat this condition therapeutically. The proposed mechanisms of CsA are twofold: immunomodulation and anti-inflammation. Immunomodulatory activity of CsA is achieved by its selective inhibition of the signal transduction cascade of inflammatory cytokines such as interleukin 2 and an eventual prevention of the autoimmune response.[5] The anti-inflammatory effect of CsA is through its inhibition of phosphatases.[6] Topical CsA treatment of dry eye patients has been reported to be clinically effective[7,8] and to reduce the numbers of activated lymphocytes and immune-related markers within the conjunctiva.[9,10] The efficacy of topical CsA in dry eye patients suggested that other immunosuppressive or anti-inflammatory drops would have a similar or greater beneficial effect in KCS patients.

The aims of this study are twofold: (1) to investigate whether conjunctival inflammation represents a primary event in the pathogenesis of KCS or whether it is a secondary inflammatory reaction caused by enhanced mechanical irritation as a result of surface dryness; and (2) to investigate whether other anti-inflammatory drops (corticosteroids and nonsteroidal anti-inflammatory) have similar therapeutic effects.

METHODS

THE STUDY WAS COMMENCED AS A SINGLE-SITE, PROSPECtive, randomized, and single-masked clinical trial. Informed consent was obtained from all patients, and the research was begun after obtaining approval from the Institutional Review Board of the Louisiana State University Health Sciences Center. The research was carried out according to the tenets of the Declaration of Helsinki. All study medications were dispensed in coded bottles. The examiner (A.M.A.) was masked as to the medication used by the patients. Thirty-two KCS patients with or without Sjögren syndrome were included in the study. All patients were at least 21 years of age. Inclusion criteria for patients included Schirmer test (without anesthesia) of 7 mm in 5 minutes or less in at least one eye; mild superficial punctate keratitis defined as a corneal punctate fluorescein score of +1 in either eye (scale 0 [none]–3 [severe]); and one or more moderate dry eye related symptom including itching, burning, blurred vision, foreign body sensation, dryness, photophobia, and soreness or pain.

Patients were excluded from the study if they had eye injury, infection, nondry eye ocular inflammation, trauma, or surgery within the previous 6 months; received concurrent treatment that could interfere with the interpretation of the study results (systemic corticosteroids, immunosuppressive therapy, and so on); had an uncontrolled disease or significant illness; or were pregnant or lactating. Postmenopausal patients who were on hormonal replacement therapy were also excluded. Before initialization of the study, the patients were instructed not to use any topical or systemic medication for at least 1 week.

On day 0, Schirmer test, tear breakup time, fluorescein and rose bengal staining examinations were performed on both eyes of all patients. The Schirmer test was performed after corneal staining, because it may affect the staining pattern of the cornea with either fluorescein or rose bengal. We used the previously described scoring system for rose bengal and fluorescein staining.[11] We also obtained symptom severity scores from patients[12] who were instructed to grade their symptoms averaging the symptom severities for both eyes. The results of the Schirmer test and rose bengal and fluorescein staining scores were evaluated separately from right and left eyes.

Impression cytology specimens were obtained from the right eyes of all patients on day 0, day 15, and day 30 to avoid statistical comparison between different eyes in different periods. For this purpose, Whatman nitrocellulose filter paper (cat no: 7195004, Whatman Int. Ltd., Maidstone, UK) was used. The paper was cut into strips of approximately 6 × 15 mm, which were pressed against the temporal bulbar conjunctiva for 5 seconds and removed.

Patients were randomized to three groups according to a computerized list generated by the LSU Eye Center biostatistician. Group 1 patients were treated with a preservative-free topical artificial tear substitute (ATS; Refresh, Allergan Inc., Irvine, California, USA) four times a day in both eyes. Group 1 patients did not receive any medication besides Refresh. Groups 2 patients were treated with topical nonsteroidal anti-inflammatory drug (NSAID) eye drops, flurbiprofen (Ocufen, Allergan Inc. Irvine, California, USA) four times a day plus ATS four to eight times a day in both eyes. Group 3 patients were treated with topical corticosteroidal drops (TSD) FML (Allergan Inc., Irvine, California, USA) four times a day plus ATS four to eight times a day in both eyes. The patients were instructed to discuss their medications only with the study coordinator and not with the examiner.

The impression cytology sample strips were cut into three pieces: one piece was used for periodic acid–Schiff (PAS) staining; one was used for HLA-DR (Monoclonal Mouse Anti Human HLA-DR, Alpha-Chain Clone TAL 1B5 Code No. M0746 Lot068 DAKO); and the other for Apo 2.7 (Monoclonal Antibody Apo 2.7 Cat. No: 2087 Immunotech) staining. The strip samples were placed separately cell side down on gelatin-coated slides. The slides, with adherent nitrocellulose strips, were air dried thoroughly at room temperature, placed in 100% methanol, and washed repeatedly with methanol until the nitrocellulose totally dissolved. The slides were then wrapped in waxed papers and mailed to the laboratory in suitable containers. The immunohistochemistry techniques reported by Bales and Durfeen[13] were used.

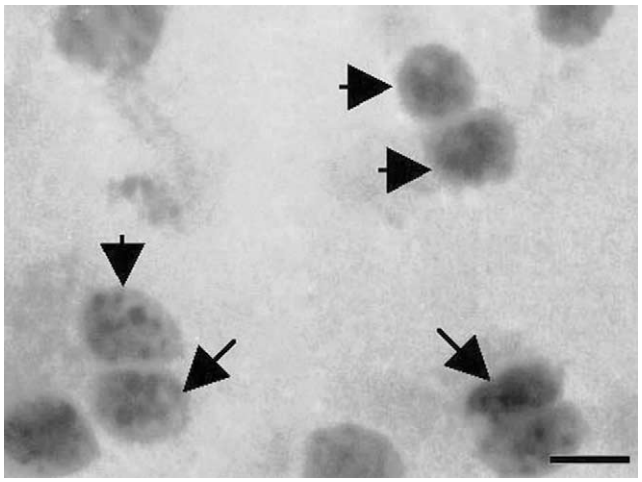

FIGURE 1. An impression cytology specimen from a patient with pretreatment period. The monoclonal antibody (anti human HLA-DR) stains cell membranes in a granular manner, as evidenced by this photomicrograph. Arrows indicate the cells stained positively (bar = 25 μ).

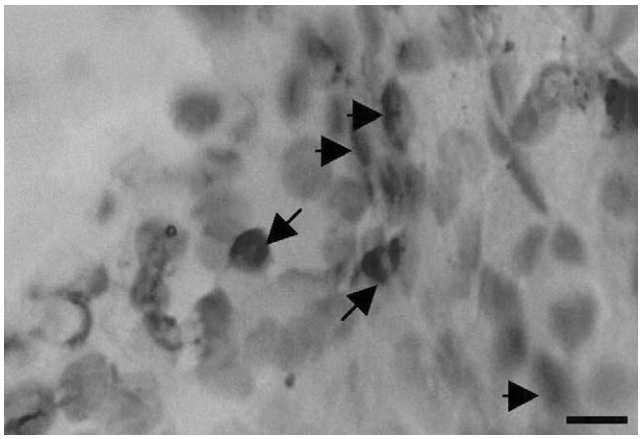

FIGURE 2. An impression cytology specimen stained with antihuman Apo 2.7 after treatment with nonsteroidal anti-inflammatory drops plus artificial tear substitute. Apo 2.7 is a specific apoptosis marker, and anti-Apo 2.7 antibody stains cell membranes of reactive cells. Arrows indicate positive cells (bar = 25 μ).

Negative and positive control slides were run with each sample. The staining pattern of goblet cells of colon mucous epithelium was used as control to PAS staining. Similarly, the staining pattern of human tonsil specimen was used as control to HLA-DR and the staining pattern of the basal cell layers of squamous epithelium was used as control to Apo 2.7 staining. The slides were viewed and photographed using a Zeiss Photomicroscope III (Zeiss, Oberkochen, Germany). To standardize data collection, four fields on each sample were viewed using a 25× objective and photographed. Cell counts were carried out on coded slides to avoid bias. In masked fashion, two examiners quantified the pattern of staining photographically (Figures 1 and 2). Percentages of reactive cells were determined by counting at least 200 cells in each specimen. Damaged cells were not considered.

Statistical analyses were performed using SPSS for Windows (Version 6.0 and 10.0, SPSS Inc., Chicago, Illinois, USA). The first comparison between and within groups on different examination days was analyzed using a two-way analysis of variance (ANOVA) test. Examination days and groups were chosen as independent variables or factors, and the other parameters were chosen as dependent variables. Within groups, changes from baseline were evaluated with a one-way ANOVA test (day was the independent variable) using the Tukey post-hoc test. The comparisons between groups on different examination points were also analyzed with a one-way ANOVA test using the Tukey post-hoc test (group was an independent variable). $P = .05$ was considered significant for all effects. The null hypothesis was that there was no difference among the treatment groups with regard to changes from baseline values. The alternate hypothesis was that there was a change.

Power was calculated to detect an among-group difference in change from baseline in symptom severity scores on day 30. The power of the study was calculated according to the given formula.[14] For a sample size of 11 patients, the power to calculate 1 grade difference was 0.64.

RESULTS

A TOTAL OF 32 PATIENTS WERE ENROLLED IN THE STUDY. Four patients were discontinued for administrative reasons. Eight patients in group 1, nine patients in group 2, and 11 patients in group 3 concluded the whole study period. None of the above discontinued patients reported adverse effects that could be related to the medications used in this study.

Group 1 contained five female and three male patients (mean age, 51.2 ± 12.4 SD). Five female and four male patients constituted group 2 (mean age, 46.67 ± 8.66 SD). Group 3 comprised seven female and four male patients (mean age, 57.6 ± 12.4 SD).

At the beginning of the study (day 0), no significant difference was detected between groups in terms of any parameters studied.

We did not observe any complication that could be linked to the study medications during treatment period.

Significant differences between groups, days, and mean effect were observed in terms of symptom severity scores (mean effect, $P = .01$; groups, $P = .01$; days, $P = .09$; two-way ANOVA). Group 3 patients had significantly less symptom severity scores both on days 15 and 30 compared with groups 1 and 2 patients ($P = .02$ and $P = .03$ for day 15 comparisons, and $P = .03$ and $P = .03$ for day 30 comparisons). When comparisons were made between the

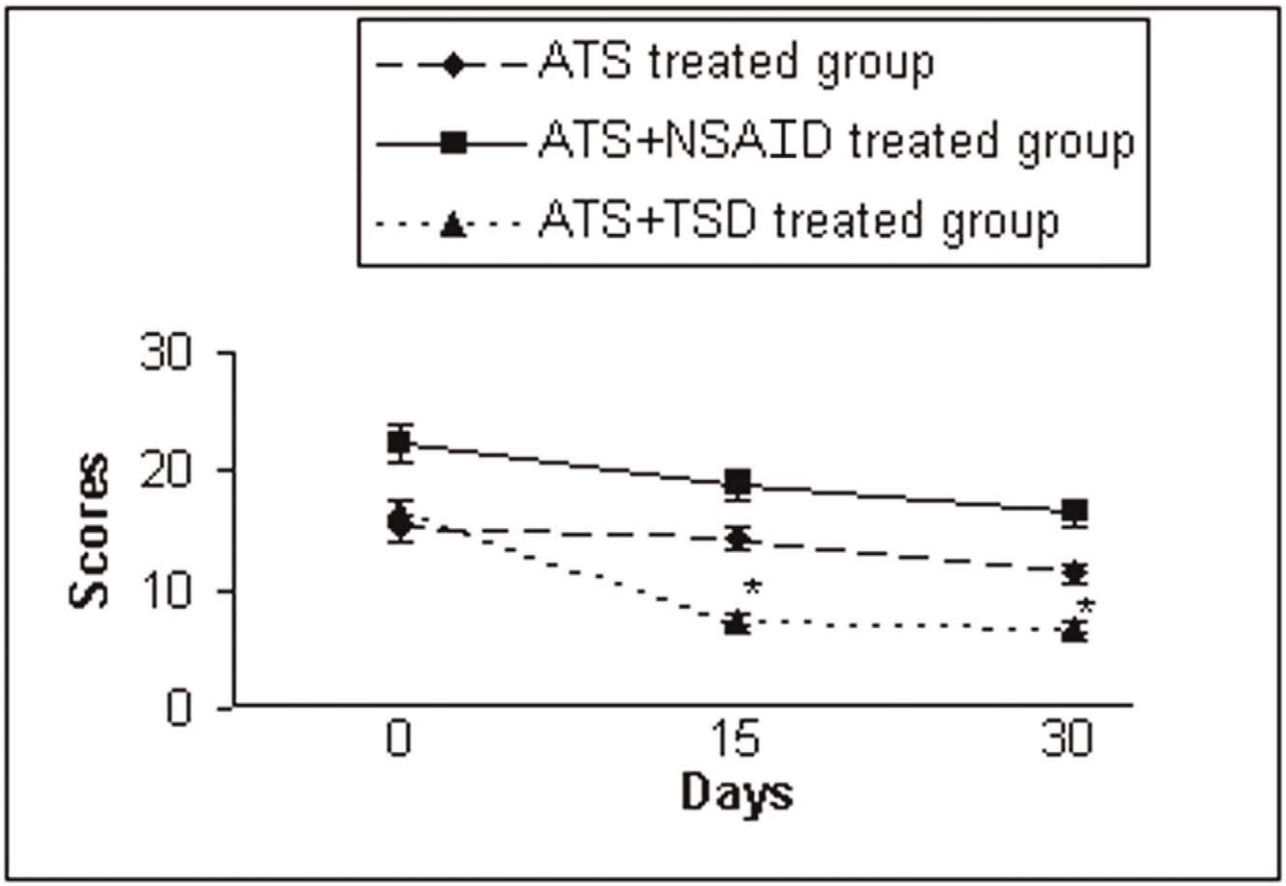

FIGURE 3. Change from baseline in symptom severity scores. *Significantly different from baseline value (all P = .000 both for days 15 and 30; P = .003 on day 15 for ATS+TSD treated group, and P = .002 on day 30 of ATS+TSD treated group). ATS = artificial tear substitute; NSAIDs = nonsteroidal anti-inflammatory drops; TSD = topical corticosteroidal drops.

treatment days in particular groups, no significant difference was found in groups 1 and 2 patients. However, a significantly less symptom severity score was detected on day 30 compared with day 0 in group 3 patients ($P = .02$). Comparison between days 0 and 15 also gave a significant result ($P = .03$). Other comparisons within and between groups did not differ significantly (Figure 3).

Similarly, when rose bengal staining of right eyes was compared between groups, significant differences were obtained in terms of main effect ($P = .03$) and group comparison ($P = .04$), but no significant difference was observed in terms of day comparison ($P = .07$) (two-way ANOVA). Although there was no significant difference observed between groups on day 15, group 3 patients had significantly lower rose bengal staining scores compared with group 2 patients on day 30 ($P = .046$), but comparison between groups 2 and 3 did not show a significant difference. In group 3 patients, the mean rose bengal staining score on day 30 was significantly lower than that of day 0 ($P = .01$), and the mean score on day 15 was also lower than that on day 0 ($P = .02$). However, comparison between days 15 and 30 was not significantly different. There was a significant difference between days 0 and 15 in group 2 patients ($P = .007$). However, other comparisons within and between groups did not give any significant difference (Figure 4). Left eye comparisons between groups gave similar results.

Comparison between groups in terms of fluorescein staining patterns of right eyes was significantly different in terms of groups ($P = .019$), but no significant difference was observed in terms of days ($P = .074$) or main effect ($P = .092$). Group 3 patients had a significantly lower fluorescein staining score compared with group 2 patients on day 30 ($P = .017$). In group 2 patients, the fluorescein staining score was significantly lower on day 15 compared with day 0 ($P = .017$). Similarly, comparisons between days 0 to 15 and 0 to 30 gave significant results in group 3 patients ($P = .018$ and 0.016, respectively). Other comparisons within and between groups did not differ significantly (Figure 5). Comparison between left eyes among fluorescein staining pattern gave similar results.

In impression cytology examinations, comparison between groups in terms of PAS staining gave a significant result among group comparison ($P = .003$) and main effect

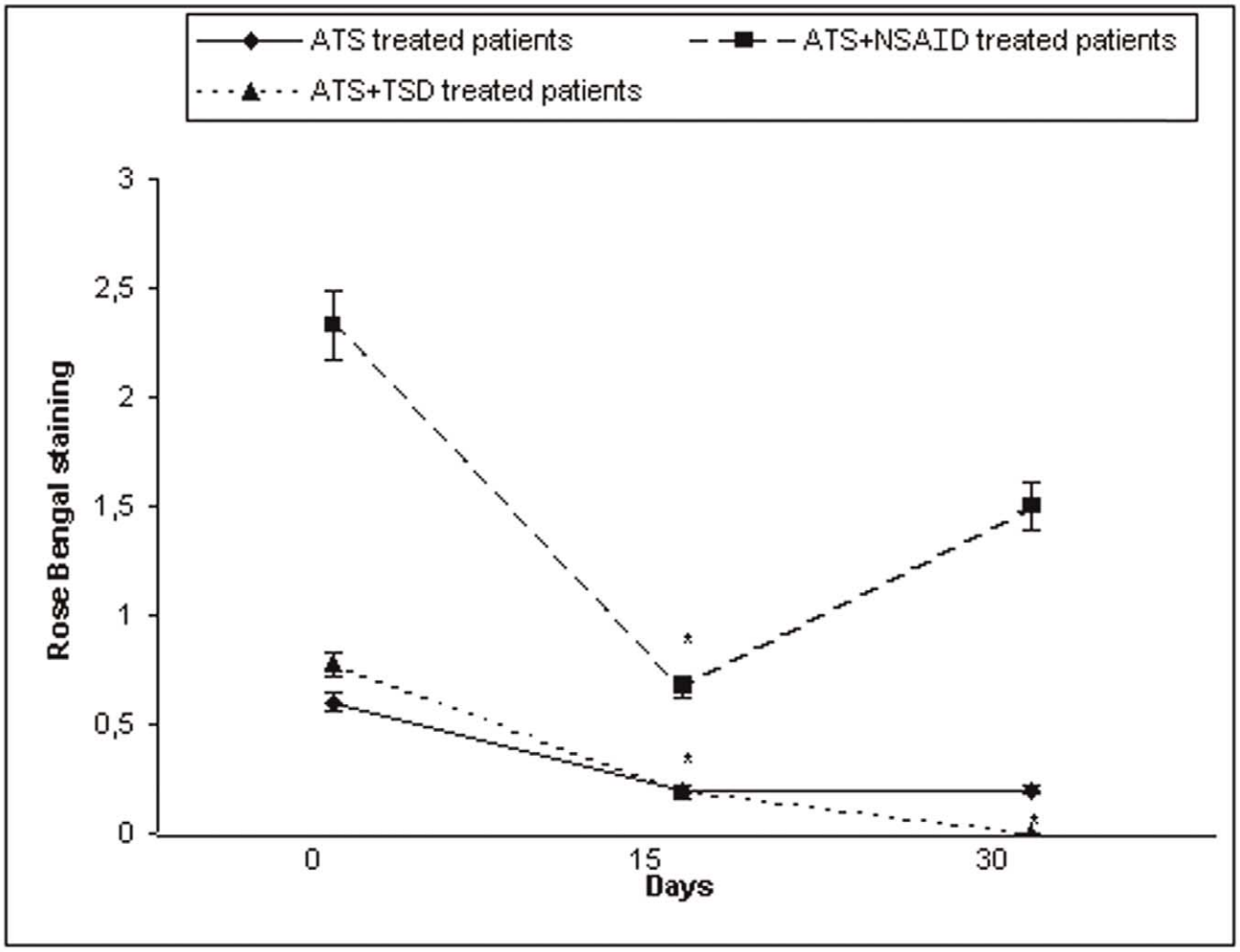

FIGURE 4. Change from baseline in rose bengal staining of right eye. *Significantly different from baseline value ($P = .007$ on day 15 of ATS+NSAIDs group; $P = .02$ on day 15 of ATS+TSD group; $P = .01$ on day 30 of ATS+TSD group). ATS = artificial tear substitute; NSAIDs = nonsteroidal anti-inflammatory drops; TSD = topical corticosteroidal drops.

($P = .012$), but no significant difference could be observed between day comparisons. On day 15, group 3 patients had a significantly greater number of PAS+ cells compared with both groups 1 and 2 patients ($P = .034$ and $P = .028$, respectively). Similar results were obtained on day 30 comparisons. On day 30, the mean percentage of PAS+ cells in group 3 patients was significantly higher than in both groups 1 and 2 patients ($P = .000$ and $P = .001$, respectively). In group 3 patients, the mean percentage of PAS+ cell was significantly higher on day 30 than both on days 0 and 15 patients ($P = .01$, $P = .02$). Other comparisons within and between groups were not significant (Figure 6).

Human leukocyte antigen (HLA) staining comparison between groups also revealed significant differences in terms of group comparison ($P = .046$), but no significant difference could be detected in terms of either days or main effect. On day 15, group 3 patients had significantly lower HLA+ cells compared with both groups 1 and 2 patients ($P = .032$ and $P = .042$, respectively). On day 30, group 3 patients had a significantly less HLA-DR+ cells compared with groups 1 and 2 ($P = .024$ and $P = .033$, respectively). In group 3 patients, mean HLA-DR+ cells on day 30 were significantly lower than that in both groups 1 and 2 on days 0 and 15 ($P = .042$ and $P = .038$, respectively). Other comparisons within and between groups did not reveal any significant difference (Figure 7).

No significant differences at any examination point (Figure 8) were found with Schirmer test values and the percentage of Apo 2.7+ cells in impression cytology specimens.

DISCUSSION

THE MOST IMPORTANT RESULTS OF THIS STUDY WERE that treatment with TSD significantly improved the ocular

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