K-13182, and the cellular adhesion of U-937 cells to HUVECs was measured thereafter. As shown in Fig. 1c, the adhesion of U-937 to TNF-α-stimulated HUVECs was inhibited significantly by the treatment of K-13182 in a dose-dependent manner ($P < 0{\cdot}01$ at 0·1, 0·3, 1 and 3 μM compared to absence of K-13182, Dunnett's multiple comparisons). The observed IC_{50} for the cellular adhesion was 0·14 μM. Ten μM of K-13182 had no toxic effect on U-937 cells (data not shown).

K-13182 inhibited cellular adhesion and the expression of VCAM-1 in a murine endothelial cell line

We further confirmed the effects of K-13182 on a murine endothelial cell line. We used MAECs that had been established in our laboratory. Expression of VCAM-1 on MAECs was up-regulated in response to TNF-α stimulation and they retained cellular adhesion activity with WEHI-3 [12]. Stimulation with 10 ng/ml TNF-α showed a maximum VCAM-1 expression after 24 h (data not shown) and reached a plateau thereafter. We therefore decided to use a dose of TNF-α (10–30 ng/ml) for later experiments.

The expression of VCAM-1 on TNF-α-stimulated MAECs was significantly down-regulated by K-13182 in a dose-dependent manner ($P < 0{\cdot}001$ at 0·01, 0·03 and 0·1 μM compared to absence of K-13182, Dunnett's multiple comparisons, Fig. 2a). The IC_{50} for VCAM-1 expression in MAECs was 0·05 μM. Cell viability was assessed by the trypan blue dye exclusion test, and no cytotoxicity of K-13182 to MAECs was observed. K-13182 (0·3 μM) also suppressed the VCAM-1 expression that was up-regulated by 10 ng/ml of TNF-α at the transcriptional level (Fig. 2c). There was more significant down-regulation of VCAM-1 mRNA in HUVECs and the concentration of K-13182 was 1 μM in HUVECs and 0·3 μM in MAECs. However, 1 μM of K-13182 did not show a significant effect compared with 0·3 μM of K-13182 in the RT–PCR in MAECs (data not shown).

To assess whether K-13182 affects mononuclear cellular adhesion with activated mouse endothelium, a cellular adhesion assay was performed using WEHI-3 and TNF-α-stimulated MAECs in the presence of K-13182. The adhesion of WEHI-3 to TNF-α-stimulated MAECs was inhibited significantly by the treatment with K-13182 in a dose-dependent manner ($P < 0{\cdot}05$ at 0·1 μM compared

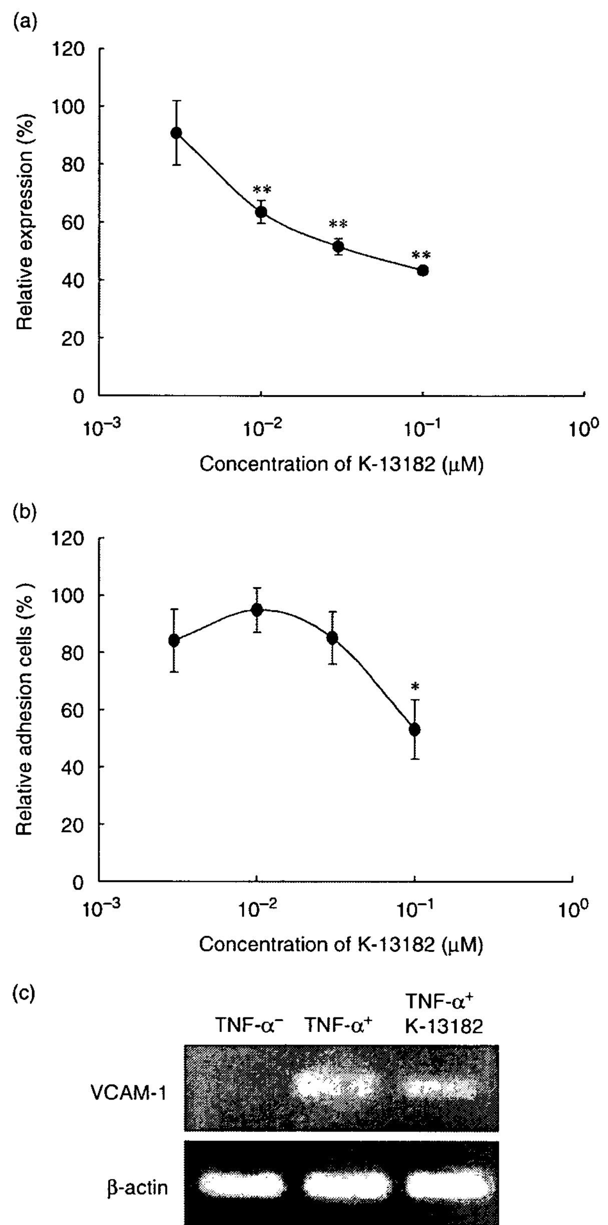

Fig. 2. Mouse vascular cell adhesion molecule-1 (VCAM-1) expression and mononuclear cell/endothelial cell adhesion. (a) Effects of K-13182 on the expression of VCAM-1 in mouse aortic vascular endothelial cell lines (MAECs). MAECs were stimulated with tumour necrosis factor (TNF)-α in the presence of K-13182 for 4 h. The expression of VCAM-1 was then investigated by cell enzyme-linked immunosorbent assay (ELISA). Inhibitory concentration (IC_{50}) was 0·05 μM. Data are expressed as percentage of control expression (without K-13182) and represented as mean ± s.d. of triplicate samples. Statistical analysis was made using Dunnett's multiple comparisons compared to absence of K-13182, $^{**}P < 0{\cdot}001$.
(b) WEHI-3/MAECs adhesion assay. MAECs were pretreated with K-13182 (0–1·0 μM) overnight and stimulated with TNF-α for 4 h. Adhesion of fluorescent-labelled WEHI-3 cells was analysed by fluorescent spectrophotometer. IC_{50} was 0·10 μM. Data are expressed as percent adhesionage of cells added and represented as mean ± s.d. of triplicate samples. Statistical analysis was made using Dunnett's multiple comparisons compared to absence of K-13182, $^{*}P < 0{\cdot}05$.
(c) Effects of K-13182 on VCAM-1 mRNA expression in MAECs. MAECs were stimulated with 10 ng/ml TNF-α in the presence of 0·3 μM of K-13182 for 4 h. The expression of VCAM-1 was then investigated by reverse transcription–polymerase chain reaction (RT–PCR).

to absence of K-13182, Dunnett's multiple comparisons, Fig. 2b). The IC_{50} for cellular adhesion assay was 0·10 μM.

Administration of K-13182 into NOD mice

The NOD mouse has been proposed as a valuable animal model for SS in humans, because NOD mice develop spontaneously mononuclear cell infiltration into the lacrimal and submandibular glands. Although diabetes developed only in female NOD mice, lymphocyte infiltration into the lacrimal glands was detected in male mice [18]. On the other hand, the destruction of submandibular glands was seen in female NOD mice [19]. Thus, we examined the *in vivo* therapeutic effects of K-13182 in male NOD mice as a murine model of lacrimal gland destruction. Body weight was monitored before (4·5 weeks of age) and after (16 weeks of age) administration of K-13182. Body weight increased in a time-dependent manner in both groups, and no statistical difference was detected between control and K-13182-treated mice at both 4·5 and 16 weeks of age (Fig. 3a). Side effects were not observed during administration of K-13182. In order to examine the anti-inflammatory effect of K-13182 in the lacrimal glands, they were analysed histologically after administration of K-13182. We evaluated their inflammatory lesions by the number of infiltrated mononuclear cells or inflammation score at 12 or 16 weeks of age, as described in the Materials and methods section. The number of infiltrated mononuclear cells in K-13182-treated mice was decreased significantly compared to control mice at 12 weeks of age ($P < 0{\cdot}05$, Student's *t*-test). At 16 weeks of age it was also slightly decreased; however, statistical difference was not detected (Fig. 3b). So far, we do not have an explanation for this discrepancy between 12 and 16 weeks. Further analysis, including the subset of infiltrated lymphocytes, may be useful to analyse this. The inflammation score was slightly decreased in K-13182-treated mice; however, no statistical difference was detected at 12 and 16 weeks of age (data not

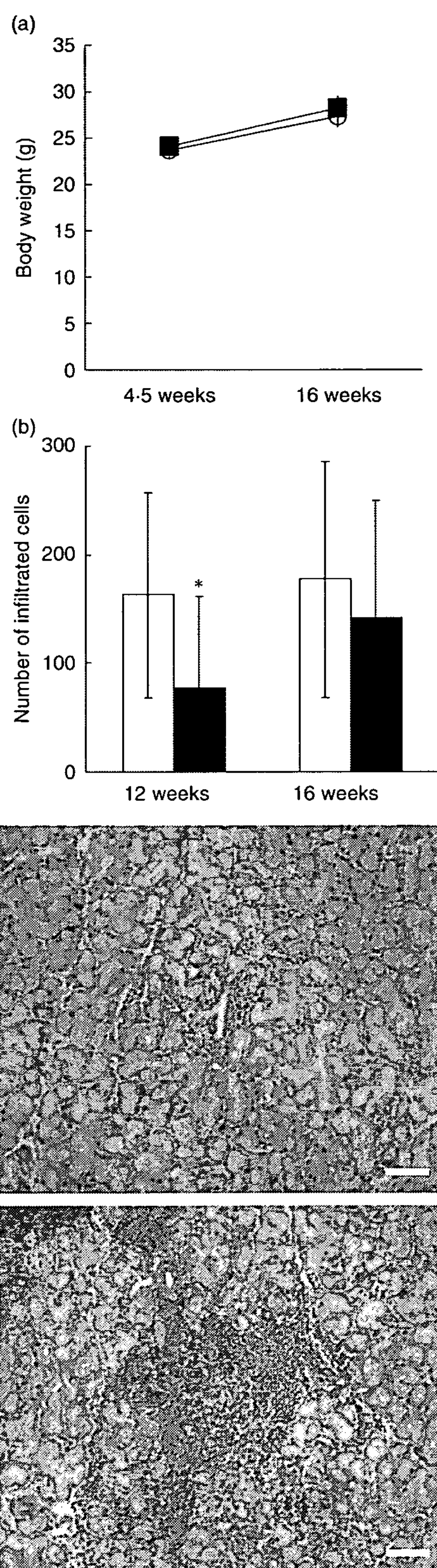

Fig. 3. Effects of *in vivo* administration of K-13182 in murine SS model. (a) The body weight of each mouse was measured at 4·5 weeks of age (control mice: open circle: $n = 5$; K-13182-treated mice: black square: $n = 5$) and at 16 weeks of age (control mice: $n = 10$; K-13182-treated mice: $n = 11$). Data are represented as mean ± s. d. of each time-point. (b) The number of infiltrated mononuclear cells in lacrimal glands at 12 weeks of age (control mice: $n = 12$; K-13182-treated mice: $n = 11$) was decreased significantly in K-13182-treated mice. Statistical difference was not detected at 16 weeks of age (control mice: $n = 14$; K-13182-treated mice: $n = 15$). The white and black bars indicate control mice and K-13182-treated mice, respectively. Data are represented as mean ± s.d. of each time-point. Statistical analysis was made using the Student's *t*-test, $^*P < 0{\cdot}05$. Representative histological features showing severe lesions in the lacrimal glands in (c) control mice but not in (d) K-13182-treated mice. Original magnification ×200, scale bar 20 μm.

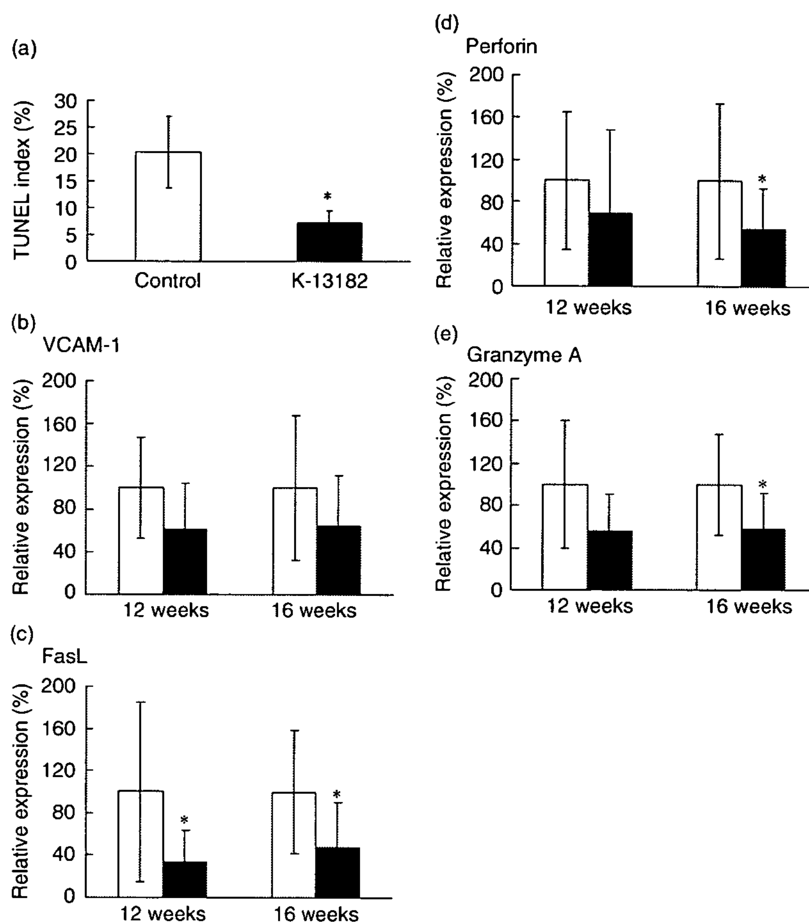

Fig. 4. Down-regulation of inflammatory molecules and apoptosis in K-13182-treated mice. (a) Apoptosis in K-13182-treated mice lacrimal glands was analysed at 12 weeks of age by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay and the percentage of TUNEL-positive cells (TUNEL index) was measured. Data were analysed in three fields per one section, and were expressed as mean ± s. d. in four (control mice) or three (K-13182-treated mice) examined in each group. Down-regulated (c) FasL; (d) perforin; (e) granzyme A mRNA in K-13182-treated mice was examined at 12 and 16 weeks of age with real-time polymerase chain reaction (PCR). (b) Vascular cell adhesion molecule-1 (VCAM-1) was slightly down-regulated but statistical significance was not detected. rRNA is the internal control for the reverse transcriptase–PCR. The white and black bars indicate control mice and K-13182-treated mice, respectively. Statistical analysis was performed using Student's *t*-test, $^*P < 0{\cdot}05$.

shown). Representative histological features in the lacrimal glands are shown in Fig. 3c,d. These results revealed that treatment with oral administration of K-13182 (30 mg/kg/day) was effective in preventing the infiltration of mononuclear cells in the lacrimal glands of the SS model mice.

Tear secretion was also measured to analyse whether an anti-inflammatory effect led to the recovery or prevention of lacrimal gland dysfunction. Tear secretion was decreased significantly in control mice (34·7% decrease, $P < 0{\cdot}05$, Student's *t*-test) at 16 weeks of age compared to 4·5 weeks of age, while such a significant decrease was not shown in K-13182-treated mice at 16 weeks of age compared to 4·5 weeks of age (22·9% decrease, statistical difference was not detected). As tear secretion is decreased spontaneously in male NOD mice [20], this result demonstrated that K-13182 showed the preventive effect for tear decrease. As described previously, male NOD mice show mononuclear cell infiltration into only lacrimal glands; we therefore describe here the effect of K-13182 on the lacrimal glands. However, the effect of K-13182 on the pancreas and submandibular glands should be proved for further characterization of this compound.

Inhibition of apoptosis and apoptosis-related genes in lacrimal glands

Apoptosis of the acinar and ductal epithelial cells of the lacrimal glands has been proposed as a possible mechanism responsible for the impairment of secretory function in NOD mice [20,21]. In our study, decreased infiltration of mononuclear cells was demonstrated in K-13182-treated mice, as described above. From our results, we investigated the apoptosis in the lacrimal glands of NOD mice by TUNEL assay. Interestingly, the percentage of apoptotic cells was decreased in the lacrimal glands of K-13182-treated mice compared to control mice at 12 weeks of age (Fig. 4a; $P < 0{\cdot}05$, Student's *t*-test). In order to analyse the further anti-inflammatory mechanism of K-13182 in lacrimal glands, mRNA levels of FasL, perforin, granzyme A and VCAM-1 were measured by quantitative PCR. The mRNA of FasL ($P < 0{\cdot}05$, Fig. 4c) at 12 weeks of age, and FasL ($P < 0{\cdot}05$, Fig. 4c), perforin ($P < 0{\cdot}05$, Fig. 4d) and granzyme A ($P < 0{\cdot}05$, Fig. 4e) at 16 weeks of age were reduced significantly in K-13182-treated mice compared with control mice (Student's *t*-test). VCAM-1 was down-regulated slightly in K-13182-treated mice; however, statistical significance was not detected at 12 and

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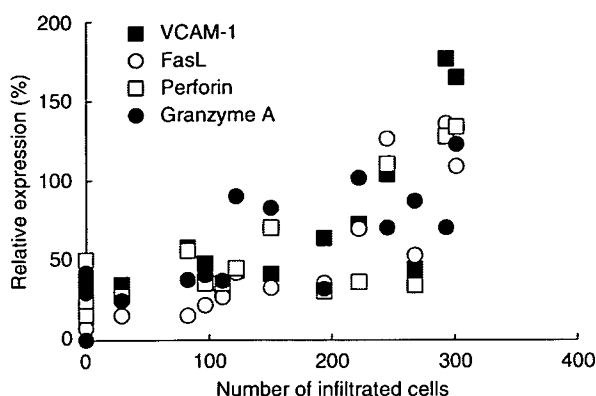

Fig. 5. Correlation coefficients between the number of infiltrated mononuclear cells and the results of quantitative polymerase chain reaction (PCR). Correlation coefficients (R^2) between the number of infiltrated mononuclear cells and the analysed gene mRNA levels were 0·59 [■: vascular cell adhesion molecule-1 (VCAM-1)], 0·74 (○: FasL), 0·46 (□ perforin) and 0·59 (●: granzyme A).

16 weeks of age (Fig. 4b). Correlation coefficients between the number of infiltrated mononuclear cells and the results of quantitative PCR were calculated to investigate the relation between the inflammation and expression of analysed genes in the lacrimal glands. As shown in Fig. 5, the expression of VCAM-1, FasL, perforin and granzyme A was proportional to the infiltrated mononuclear cell numbers at 16 weeks of age. Correlation coefficients (R^2) to infiltrated mononuclear cell number was 0·59 for VCAM-1, 0·74 for FasL, 0·46 for perforin and 0·59 for granzyme A.

Discussion

In the present study, our purpose was to assess the therapeutic effect of K-13182 as a therapeutic agent for SS *in vitro* and *in vivo*.

Regarding the inhibition of cellular adhesion of K-13182 between endothelial cells and mononuclear cells, as shown in *in vitro* experiments (Figs 1 and 2), K-13182 inhibited VCAM-1 expression in HUVECs and MAECs. In addition, cellular adhesion with endothelial cells and mononuclear cells was prevented in both human and murine co-culture assay systems. These results indicated that K-13182 retains the ability to inhibit mononuclear infiltration into the inflammatory region via endothelial cells, and the therapeutic effect shown in the *in vivo* analyses might be mediated by the prevention of cellular adhesion by K-13182.

Considering the effect of K-13182 on the VCAM-1 expression and cellular adhesion *in vitro*, IC_{50} for VCAM-1 expression was 0·019 μM (HUVECs) and 0·05 μM (MAECs), while IC_{50} for cellular adhesion was 0·14 μM (HUVECs) and 0·10 μM (MAECs). These results suggest that adhesion molecules other than VCAM-1 also contribute to cellular adhesion. Nakao *et al.* have shown that ICAM-1/β2 integrin also participated in the adhesion of U-937 cells on HUVECs [22]. In the VCAM-1 gene promoter region, functional transcription factor binding motifs have been reported, including nuclear factor (NF)-κB, interferon regulatory factor, AP-1 and GATA [23–27], while the ICAM-1 gene promoter also has SP-1, NF-κB and AP-1 binding sites; however, it does not contain the GATA binding motifs [28]. Taken together, K-13182 might have inhibited VCAM-1 but not ICAM-1 expression through the regulation of GATA in HUVECs and MAECs. Human VCAM-1 promoter region contains two conserved consensus binding sites for the GATA family, while the murine VCAM-1 promoter contains one site [29,30]. This number of GATA binding motifs may explain the smaller IC_{50} in HUVECs than in MAECs in VCAM-1 expression assays and cellular adhesion assays. K-13182 was developed from a compound (K-7174) that had shown an inhibitory effect for the GATA family [15]. However, details of the mechanisms regarding the inhibition of GATA and characteristics of K-13182 and K-7174 should be investigated in future study.

Following 12-week administration of K-13182, decreased infiltration of mononuclear cells to lacrimal glands was shown by histological analysis (Fig. 3b) and the preventive effect of K-13182 for tear decrease was also confirmed. These anti-inflammatory effects of K-13182 might be mediated by the down-regulation of VCAM-1 expression and mononuclear cell adhesion, considering the *in vitro* analyses (Figs 1 and 2). However, a statistically significant difference was not detected in the VCAM-1 expression in lacrimal glands of NOD mice (Fig. 4b). This discrepancy between the *in vitro* and *in vivo* experiments may be caused by the sample; we used endothelial cells in the *in vitro* experiment, while lacrimal glands used for quantitative PCR included various kind of cells. This sampling may lead to a slight suppression of VCAM-1 in K-13182-treated mice. Furthermore, the smaller IC_{50} of K-13182 for MAECs compared with the IC_{50} for HUVECs discussed above and the results of the RT–PCR (Figs 1d and 2c) may explain partly the low therapeutic efficacy including VCAM-1 mRNA expression in lacrimal glands in a mouse model. Although further investigation is needed to find in which cells K-13182 is effective in lacrimal glands, K-13182 is considered to contribute to the down-regulation of VCAM-1 expression and mononuclear cell infiltration in lacrimal glands because high correlation coefficients were detected between VCAM-1 expression and infiltrated cell number in K-13182-treated mice (Fig. 5).

Considering the decreased mononuclear infiltration in K-13182-treated mice lacrimal glands and proposed mechanism of apoptosis in NOD mice [20,21], we speculated that the apoptosis suppression that may be induced due to the reduced mononuclear cell infiltration via cell adhesion molecules including VCAM-1. TUNEL assay showed the decreased number of apoptotic cells in K-13182-treated mice lacrimal glands. Furthermore, quantitative PCR analysis in K-13182-treated mice lacrimal glands also

demonstrated the down-regulation of FasL, perforin and granzyme A, which are involved in SS [31–34]. Fas/FasL-mediated tissue destruction in SS lacrimal glands were also reported [31,32]. Granzyme A is a serine protease that is contained in the granules of activated lymphocytes and plays a role in the destruction of the SS lacrimal glands [33], and perforin was also reported to be expressed in the lacrimal glands of SS [34]. Taken together with our results, the prevention of the tear decrease in K-13182-treated mice can be explained partly by the suppression of apoptosis and molecules such as FasL, perforin and granzyme A that may be decreased due to the reduced mononuclear cell infiltration via cell adhesion molecules including VCAM-1.

Saito *et al.* determined that several cell adhesion molecules are involved in the lymphoid cell infiltration of salivary and lacrimal glands in SS patients [10]. Furthermore, previous studies have demonstrated that neutralizing monoclonal antibodies (mAb) of cell adhesion molecules prevented the development of experimental autoimmune encephalomyelitis in the murine model [35,36]. Therefore, the inhibition of signal transduction pathways involved in lymphocyte adhesion is also valuable in treating these autoimmune diseases. VCAM-1 plays a critical role in inflammatory reactions, from the original recruitment of leucocytes through successive steps in the continuing process [11]. In fact, VCAM-1/VLA-4 has been studied as a target for a variety of autoimmune diseases. The humanized mAb to alpha4 integrin, natalizumab, was approved recently in the United States for the treatment of relapsing multiple sclerosis [37]. Neutralizing mAb to VCAM-1 reduced clinical severity in collagen-induced arthritis in mice [38], and peptide inhibitor for VCAM-1 also prevented airway hyperresponsiveness in sheep [39]. Therefore, specific inhibitors of VCAM-1 induction in endothelial cells have been sought actively to develop a new therapeutic approach that would not elicit severe side effects in the case of the treatment of atherosclerosis or various chronic inflammatory disorders, including SS [40].

We have demonstrated here the potent effect of K-13182 in SS model mice, which might be caused by the preventive effect of cellular adhesion mediated by VCAM-1. Our results are consistent with a former report that showed antibodies against VCAM-1 almost completely blocked lymphocyte migration from the blood into inflamed lacrimal glands in NOD mice [18]. K-13182 showed similar effects on VCAM-1 expression and mononuclear adhesion both in murine and human endothelial cells. In this report, K-13182 was administered prior to disease onset for the prevention of the disease, therefore the therapeutic effect of K-13182 should be analysed further. However, from our results, K-13182 is a promising candidate for a SS therapeutic reagent.

Acknowledgements

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Noninvasive Interference Tear Meniscometry in Dry Eye Patients With Sjögren Syndrome

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• PURPOSE: To compare noninvasive tear meniscus height (NI-TMH) using a tear interference device in normal subjects and dry eye patients with Sjögren syndrome (SS), and to investigate the applicability of this new method before and after the punctal occlusion procedure.

• DESIGN: Prospective case control study.

• METHODS: Tear meniscus was visualized noninvasively using a tear interference device (Tearscope plus, Keeler, Windsor, United Kingdom). Tear interference image was captured with digital video camera (SP-321, JFC Sales Plan Co, Tokyo, Japan) attached to the slit-lamp. Lower lid margin NI-TMH was measured using image analysis software. NI-TMH of 28 eyes from 17 normal subjects and 46 eyes from 27 aqueous tear deficiency (ATD) dry eye patients with SS were compared. The change of NI-TMH three weeks after the successful punctal occlusion was examined in 11 eyes of eight dry eye subjects.

• RESULTS: Tear meniscus was well visualized with the tear interference device in all cases. Lower lid margin NI-TMH was 0.22 ± 0.065 mm in normal subjects, and 0.13 ± 0.042 mm in SS subjects, respectively ($P < .0001$). After the punctal occlusion, lower lid margin NI-TMH increased significantly from 0.12 ± 0.026 mm to 0.42 ± 0.21 mm ($P = .001$).

• CONCLUSIONS: NI-TMH was substantially lower in SS subjects and also significantly improved after punctal occlusion. This method is expected to be helpful in the diagnosis and in the evaluation of the efficacy of punctal occlusion in ATD dry eyes such as SS. (Am J Ophthalmol 2007;144:232–237. © 2007 by Elsevier Inc. All rights reserved.)

TEARS ARE SECRETED FROM THE LACRIMAL GLAND and distributed by blinking to form the tear film of the ocular surface.[1–3] Tear film is responsible for wetting the ocular surface, which is the first line of defense, and is also essential for clear visual imaging.[4–6] Tears are distributed to the cul-de-sac and the exposed ocular surface area, including tear menisci.[7] Tear meniscus is a major part of the tear reservoir, which holds approximately 70% to 80% of the total tear volume of the exposed ocular surface area. Thus, tear menisci have been considered to reflect tear volume on the ocular surface, and have been considered to be important in the diagnosis of dry eye syndrome.[8]

Using a minimal amount of fluorescein staining, Mainstone and associates reported that the fluorescein-stained tear meniscus height (f-TMH) of normal subjects was 0.46 mm, and f-TMH of dry eye subjects was significantly decreased as 0.24 mm.[9] This conventional method is widely used as the most common method of tear meniscus height (TMH) measurement. Oguz and associates also reported that f-TMH of dry eye subjects using minimal fluorescein was 0.21 mm, and TMH of the same subjects measured by a noninvasive method with slit-lamp equipped with a micrometer-scale was 0.19 mm.[10] In the meantime, they pointed out that TMH of dry eye subjects sometimes could not be observed without fluorescein, since it was too low. Conventional f-TMH has been reported to be of value in the diagnosis of dry eye; however, it was limited by its invasive nature.[9,11–15]

Another method to visualize the lucent tear meniscus is to use interference phenomena. Tear interferometry is a noninvasive visualization method of the lucent tear film. The optical path difference from the reflectance at the surface of the tear lipid layer and at the interface of the tear lipid-aqueous layer causes a tear interference image, which could be clearly observed.[16–18] It has been mainly used to observe the precorneal tear lipid layer.[19–21]

In ophthalmic practice using the tear interference device (Tearscope Plus, Keeler, Windsor, United Kingdom),[22–26] not only can the precorneal tear film be observed clearly and noninvasively, but the tear film at the tear meniscus as well. Therefore, we hypothesized that noninvasive tear meniscus height (NI-TMH) could be quantified with the image analysis of tear meniscus interference image, and could be used to differentiate aqueous tear deficiency (ATD) dry eye such as Sjögren syndrome (SS) or to assess surgical efficacy after dry eye treatment by punctal occlusion. The significance of this method and findings are further discussed.

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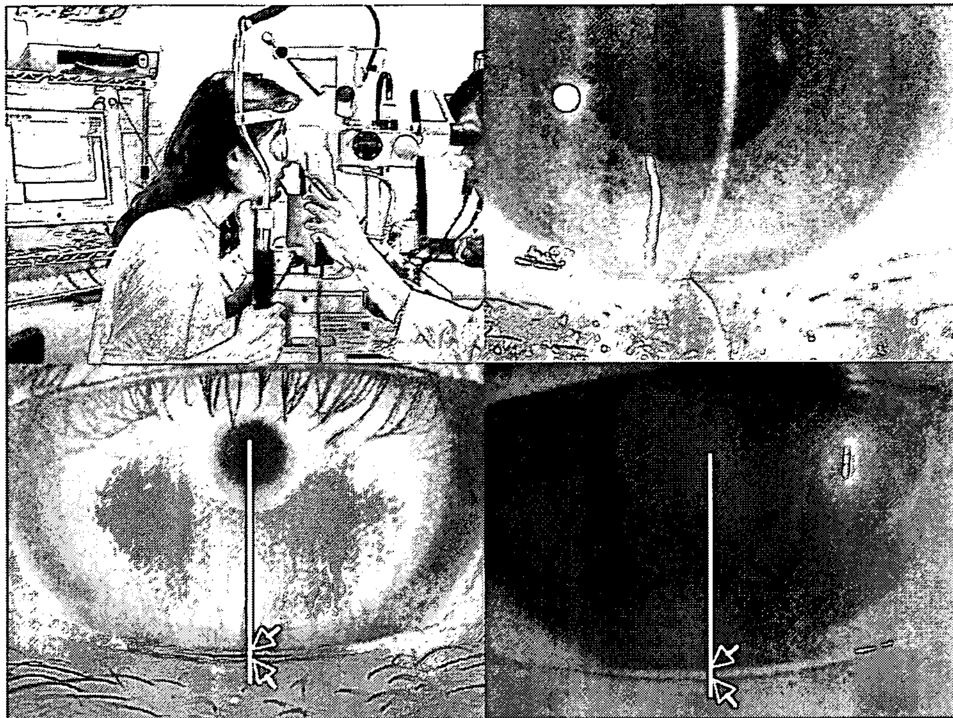

FIGURE 1. Measurement of noninvasive tear meniscus height (NI-TMH) using Tearscope Plus. (Top left) Tearscope interference device is set between the subject's eye and the slit-lamp. Precorneal tear interference image and tear meniscus interference image could be observed through the slit-lamp, and was recorded to the computer through the mounted digital video camera. Tear interference image with meniscus could also be seen on the computer screen. (Top right) Slit-lamp image of tear meniscus with diffuser light is shown. Tear meniscus of the same subject in Top left image is noninvasively visualized (Bottom left) and is also visualized with fluorescein staining (Bottom right). (Bottom left) Using image analysis software, the height of noninvasively visualized tear meniscus (between upper and lower white arrow) in central area (vertical white line) was measured. NI-TMH was quantified as 0.21 mm. Note that surface lipid layer of both tear meniscus and precorneal tear film is visualized by the tear interference device. (Bottom right) Using image analysis software, the height of fluorescein stained tear meniscus (between upper and lower white arrow) in central area (vertical white line) was measured in the same image capturing system. Fluorescein-stained tear meniscus height was quantified as 0.24 mm.

METHODS

• MEASUREMENT OF NONINVASIVE TEAR MENISCUS HEIGHT USING TEAR INTERFERENCE DEVICE: Tearscope Plus tear interference device was attached to the slit-lamp (SL130, Zeiss, Jena, Germany, magnification fixed to 12 ×, Figure 1). The tear interference image of the lower tear meniscus could be observed noninvasively when focusing at the lower lid margin. The image was captured using a high quality digital video camera (SP-321, JFC Sales Plan Co, Tokyo, Japan) attached through the beam-splitter of the slit-lamp and recorded using an image capturing system (P4m/MaxFile, P4 Medic Co, Kobe, Japan) in 720 × 480 pixels sized JPEG format. NI-TMH was measured using the ImageJ 1.32 image analysis software (National Institutes of Health, Bethesda, Maryland, USA). None of the subjects received any eye drop instillations at least six hours before the measurement.

As NI-TMH measurement with Tearscope Plus device has not been reported, it was compared concomitantly with conventional f-TMH in 31 eyes of 16 subjects. Nine eyes of five dry eye patients with SS (five females, mean age, 64 ± 8 years) and 22 eyes of 11 normal subjects (five males and six females, mean age, 34 ± 12 years) were measured. Initially, NI-TMH was measured with the Tearscope Plus tear interference device. Then, f-TMH was measured one minute after instillation of 2 μl of fluorescein solution with a micropipette. The images of NI-TMH and f-TMH were recorded and measured using exactly the same set-up as described above. The mean NI-TMH was 0.20 ± 0.09 mm, and f-TMH was 0.26 ± 0.11 mm. Images of the representative cases of NI-TMH and f-TMH are shown in Figure 1. The correlation between NI-TMH and f-TMH was also calculated with linear regression analysis. A significant correlation was found between NI-TMH and f-TMH ($r = .79$, $P < .0001$).

• SUBJECTS AND ASSESSMENT OF TEARS AND OCULAR SURFACE: We examined a consecutive series of 27 dry eye patients with SS (46 eyes, all female, mean age, 62 ± 10 years), as well as 17 normal subjects (28 eyes, all female, mean age, 52 ± 16 years). SS patients were diagnosed

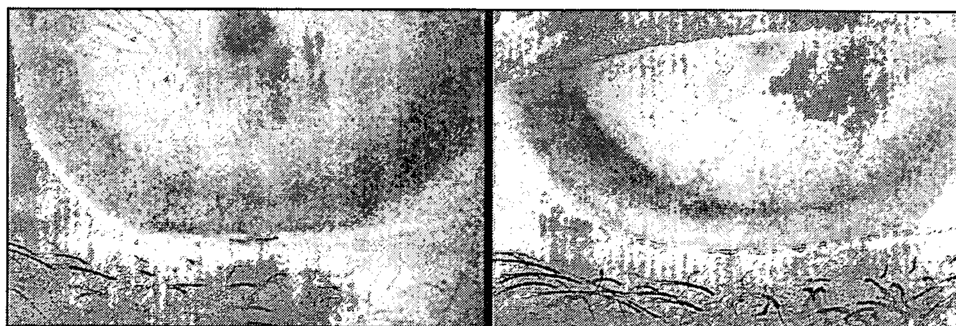

FIGURE 2. Noninvasive tear meniscus height (NI-TMH) between normal subjects and dry eye with Sjögren syndrome (SS). (Left) NI-TMH of a representative normal subject (0.28 mm). (Right) NI-TMH of a representative dry eye subject with SS (0.094 mm).

FIGURE 3. Noninvasive tear meniscus height (NI-TMH) before and after punctal occlusion. (Left) NI-TMH of a representative dry eye patient with Sjögren syndrome (SS) before punctal occlusion (0.17 mm). (Right) NI-TMH after punctual occlusion of the same patient (0.56 mm).

according to the criteria of Fox and associates.[27] Among the SS patients, eyes with a Schirmer I test value less than or equal to 5 mm were included in the study as they were considered to have ATD dry eye according to the Japanese dry eye criteria.[28] Eyes with a history of punctal occlusion, conjunctivochalasis, corneal transplantation, or corneal perforation were excluded from the study. In addition, eyes with anterior blepharitis and infectious conjunctivitis were also excluded. No patients used contact lenses in this study.

NI-TMH was assessed as described above before any invasive procedure. After that, the cornea was examined by fluorescein staining. A 2-μl volume of preservative-free solution consisting of 1% fluorescein dye was applied to the conjunctival sac. The intensity of the actual fluorescein staining of the cornea such as superficial punctate keratopathy was rated from a minimum of zero to a maximum of three, in each upper, middle, and lower cornea. Thus, the maximum total staining score was 9.[29] Tear film break-up time (BUT) was measured three times, and the measurements were averaged.[29] 2 μl of preservative-free solution consisting of 1% Rose Bengal dye was then applied to the conjunctival sac. The intensity of rose bengal staining in the cornea and conjunctiva was recorded, with the maximum score rated as nine points.[30] The Schirmer I test was then performed to measure the tear secretion volume.[31] NI-TMH was compared between dry eye subjects and normal controls.

• CHANGE OF TEAR MENISCUS HEIGHT AFTER PUNCTAL OCCLUSION: All dry eye patients received treatment with non-preserved artificial tears, and 0.1% non-preserved hyaluronic acid eye drops as necessary for at least two months. These subjects who were refractory to this treatment protocol underwent punctal occlusion. NI-TMH was compared before and three weeks after punctal occlusion or punctal plug insertion for both superior and inferior puncta in 11 eyes of eight subjects in an additional interventional case series (eight females, mean age, 69 ± 8 years). Flex plugs (Eagle Vision, Memphis, Tennessee, USA) were used for punctal occlusion in three eyes of three subjects, and punctal cauterization using Optemp 2 (Alcon, Fort Worth, Texas, USA) was performed in eight eyes of five subjects. Tseng's method was performed in punctal occlusion surgery[32] and the operation was successful in all cases without re-canalization.

• STATISTICAL ANALYSIS: All data are shown as means ± standard deviation. The Mann-Whitney *U* test was applied to the comparison of NI-TMH, fluorescein staining, rose bengal staining, tear film BUT, and Schirmer I test between SS and normal subjects. Wilcoxon matched pairs

test was applied to the comparison before and after punctal occlusion at each examination. A level of $P < .05$ was accepted as statistically significant. Graphpad Instat 3.0 (Graphpad Software Inc, San Diego, California, USA) was used for statistical analysis.

RESULTS

THE MEAN NI-TMH IN NORMAL SUBJECTS WAS 0.22 ± 0.065 mm. On the contrary, it was significantly lower (0.13 ± 0.042 mm, $P < .0001$) in dry eye patients with SS. The representative cases are shown in Figure 2. Corneal fluorescein staining mean score was significantly lower (0.46 ± 0.64) in normal subjects compared to dry eye patients with SS (4.0 ± 2.1, $P < .0001$). Rose Bengal staining mean score was significantly lower in normal subjects (0.18 ± 0.48) compared to dry eye patients with SS (4.6 ± 1.8, $P < .0001$). Similarly, tear film BUT was 5.9 ± 3.0 seconds in normal subjects, and it was significantly longer than in dry eye patients with SS (2.3 ± 1.4 seconds, $P < .0001$). Schirmer I test result was 13.9 ± 9.4 mm in normal subjects, and it was significantly longer than in dry eye patients with SS (1.7 ± 1.5 mm, $P < .0001$).

Images of NI-TMH before and after punctal occlusion in the representative case are shown in Figure 3. The mean NI-TMH significantly increased from 0.12 ± 0.026 mm to 0.42 ± 0.21 mm after the punctal occlusion procedure ($P = .001$). NI-TMH was increased after both punctal cauterization or punctal plug insertion procedures. In addition, corneal fluorescein staining mean score significantly decreased from 4.5 ± 2.3 to 0.27 ± 0.65 ($P = .002$), tear film BUT was prolonged from 0.91 ± 0.30 seconds to 5.2 ± 2.8 seconds ($P = .001$) and the Schirmer I test result increased from 2.8 ± 2.0 mm to 6.8 ± 4.2 mm ($P = .005$). On the contrary, Rose Bengal staining mean score decreased, but not significantly, from 5.0 ± 1.7 to 2.5 ± 2.0 ($P = .06$).

DISCUSSION

IN THE PRESENT STUDY, USING THE TEAR INTERFERENCE device, tear meniscus was successfully visualized in a noninvasive manner in all cases. We showed that NI-TMH measurement could be as relevant as the conventional f-TMH measuring method in the diagnosis of dry eye syndromes, could differentiate between normal subjects and ATD dry eye patients with SS, and could help in the evaluation of the change of meniscus height after punctal occlusion.

NI-TMH was significantly lower in dry eye patients with SS (0.13 ± 0.042 mm) compared with normal controls, (0.22 ± 0.065 mm) along with higher fluorescein and rose bengal staining, shortened tear film BUT, and lower Schirmer I test result. After punctal occlusion, NI-TMH significantly increased from 0.12 ± 0.026 mm to 0.42 ± 0.21 mm along with the improvement of corneal fluorescein staining, tear film BUT, and Schirmer I test result. NI-TMH was increased after both punctal cauterization or punctal plug insertion procedures. We believe that NI-TMH accurately reflects the deficiency of tear volume on the ocular surface in ATD dry eye patients with SS.

The values of NI-TMH in this study are low compared with the previous studies on TMH.[9,11–15,33] The previous data related to TMH mainly measured with fluorescein dye. In this study, a significant correlation was found between NI-TMH and f-TMH, and NI-TMH was slightly lower than f-TMH. This was possibly because of the addition of a minimal amount of water added to the fluorescein dye. The other merit of the present method is visualization ability even when the TMH is very low. In a previous study, using direct observation of the TMH with the slit-lamp, Oguz and associates reported that tear meniscus could not be observed when it was too low in dry eye subjects.[10] Our method using interference phenomena could visualize clearly such low tear meniscus even in ATD dry eyes with SS (Figures 2 and 3). Furthermore, in the principle of tear interferometry, reflectance is ranged approximately from 2% to 6%.[17,18,34] Thus, tear interference image of tear meniscus could be visualized even in dry eye cases with lipid tear deficiency. Using optical coherence tomography in ATD dry eyes, Savini and associates recently reported that mean NI-TMH was significantly lower in patients with ATD dry eyes (0.13 ± 0.07 mm) than in the control group (0.25 ± 0.08 mm).[35] We considered that their results strongly support the relevance of our method.

Compared with fluorescein-stained tear meniscus observation, noninvasive tear meniscus observation using the interference device has one demerit in terms of the limitation in the observation area. As shown in the figures, this method could visualize frontal tear meniscus at a limited observation angle. To observe all lower and upper tear meniscus areas from the inner to outer canthi, we considered that the fluorescein staining method still has some advantages.

Recently, another tear meniscus measuring device to measure meniscus radius curvature has been reported by Yokoi and associates.[36,37] This noninvasive method, however, is not widely available yet, and we chose the Tearscope interference device for the evaluation of tear meniscus in this study. Furthermore, height and radius of tear meniscus have been reported to have a positive correlation by Yokoi's group.[10] Thus, we also believe that the measurement of the NI-TMH is important, as well as tear meniscus radius measurement.[38]

In this study, we compared NI-TMH of normal and dry eye patients with SS who are representing ATD dry eyes. In the future, NI-TMH measurement of the other dry eye subtypes such as non-SS dry eye, meibomian gland dys-

function,[39] or dry eye with only decreased tear film BUT[40] would be highly anticipated. Furthermore, observation of the upper NI-TMH using Tearscope in superior limbic keratoconjunctivitis,[41] or lid-wiper syndrome[42] would be also interesting.

As many clinicians are aware, the diagnosis of ATD dry eyes is sometimes difficult owing to the variability of the Schirmer I test results by its invasive nature. In the future, we expect that NI-TMH measurement by the tear interference device would become an established tear volume evaluation test such as the Schirmer I test.[31]

In conclusion, NI-TMH measurement using the tear interference device could be considered to have similar clinical relevance compared with conventional f-TMH measurement. Not only did this method evaluate tear aqueous volume noninvasively, but it could also indicate significantly lower NI-TMH in ATD dry eye patients with SS and, was useful for indicating the increase of NI-TMH after the punctal occlusion procedure. The difference of NI-TMH in normal and dry eye groups was considered to reflect the difference of tear volume, which is responsible for moistening and maintaining the ocular surface.

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Biosketch

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