

Preliminary Effects of Oral Uridine on the Ocular Surface in Dry Eye Patients

We designed a randomized, double blinded, 3-months controlled prospective clinical study to investigate effects of oral uridine on the ocular surface in dry eye patients. Twenty-seven patients who diagnosed as dry eye with lower than 5 mm of wetting in the Schirmer strip, with corneal epithelial erosion and who completely followed-up till 3 months were enrolled. Corneal-conjunctival fluorescein staining, non-anesthetic Schirmer test, impression cytology, and Ocular Surface Disease Index (OSDI) were evaluated in the experimental and placebo groups at the baseline, 1 and 3 months after start of medication in a double blinded manner. Fluorescein stain score of the cornea was markedly decreased in oral uridine group compared to the placebo group at 3 months after medication ($P=0.032$, Mann-Whitney U test). The Schirmer wetting score for the oral uridine group was significantly increased ($P=0.001$, Wilcoxon signed rank test) at 3 months and its difference between two groups was statistically significant ($P=0.030$, Mann-Whitney U test). OSDI scores were significantly decreased at 1 and 3 months in treatment group. Oral uridine is effective in treatment of dry eyes.

Key Words : Cornea; Dry Eye Syndromes; Uridine; OSDI

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INTRODUCTION

The dry eye syndrome is one of the most prevalent diseases of the eye that affects approximately 10% to 20% of adult population (1). Dry eye develops from multiple etiologies such as a tear deficiency, abnormal evaporation, surface inflammation and abnormal neural regulation (2). The most common medical treatments for dry eye are currently artificial tear supplementation, immune suppression with medications such as cyclosporine A (3), and hyaluronic acid (HA) (4) which promotes epithelial healing.

Recently, the role of the P2Y₂ receptor agonist on dry eye has been investigated in vitro and in vivo (5-7). Topical administration of uracil-cytosine dinucleotide denufosol (Diquafosol) is currently undergoing clinical trials for dry eye disease (8). Uridine is transformed to UTP (uridine 5-triphosphate) by uridine kinase in vivo after administration. UTP, as a P2Y₂ receptor agonist, is known to stimulate mucin secretion in vitro and in vivo in conjunctiva, which increases tear film sta-

bility and protects against desiccation of the ocular surface (9, 10).

In our previous study, we showed that topical uridine could restore the health of the ocular surface in a rabbit corneal wound and dry eye model (11). Furthermore, elevated uridine 5'-diphosphate (UDP)-glucose has been shown to contribute to an increase in the synthesis of intrinsic HA (12, 13). Hence, oral uridine may help patients with dry eye by increasing tear secretion or hyaluronic acid. In fact, oral uridine is currently used as a dietary supplement and is commercially available.

Recently, the concept of dry eye has been changed to focus on the importance of dysfunctional tears. The Delphi panel reported on the crucial role of the symptoms as well as the clinical signs for the diagnosis of the dysfunctional tear syndrome (13). Even without any sign of a damaged ocular surface, complaints of dryness or a foreign body sensation can be diagnosed as the dysfunctional tear syndrome based on the guidelines provided by the Delphi panel committee (14-16).

Therefore, the aim of the present study was to investigate

the effect of oral uridine on the ocular surface in dry eye patients, by evaluating the clinical signs and symptoms with the Ocular Surface Disease Index (OSDI).

MATERIALS AND METHODS

Patient selection

The study was approved by the institutional review board of Seoul National University Hospital and written consent was obtained from each patient before participation; the study protocol followed the guidelines of the Declaration of Helsinki. Patients who were diagnosed with bilateral dry eye syndrome, showing lower than 5 mm of wetting with the Schirmer strip, superficial punctate keratitis and no improvement despite the use of any prescribed medications for at least 2 months in their both eyes were recruited from the Seoul National University Hospital from May 2005 to May 2006. The patients were initially enrolled in a randomized, double-blinded, 3-month, controlled clinical study. 'No improvement' was defined as a case that despite previously prescribed medications, patient showed no change in their symptoms, Schirmer test and fluorescein score for more than 2 months.

Subjects were excluded if they had any of the following: dry eye caused by Stevens-Johnson syndrome or cicatricial pemphigoid, a history of intraocular surgery or corneal surgery such as LASIK, or corneal transplant before enrollment. In addition, patients with neurotrophic keratitis, ongoing ocular infection such as a significant blepharitis, *herpes* infection, or bacterial keratitis were excluded. Furthermore, if the subjects could not complete a 3-month follow-up or take their medications accurately, they were excluded.

If they had any of new systemic symptoms after taking medication such as indigestion, diarrhea, we recommended them to stop taking medications.

Treatment

Patients were randomly assigned to 3-months of treatment with oral uridine (HALUTECH plus[®], KFDA, No.2004-0060-047, 800 mg/tab, MDbioalpha, Daejeon, Korea) or placebo, 2 tablets 3 times a day. The placebo was composed of L-glutamine, lactose and crystalline cellulose which was the same color and shape as HALUTECH plus[®] but contained no uridine. HALUTECH plus[®] which was developed for degenerative arthritis, included 50 mg of uridine in its one tablet. The patients were allowed to use their all previously prescribed medications except for the hyaluronic acid eye drops for minimizing the possible confounding effects. The treatment medication or placebo was given to the patients by the medical assistant who was not involved in the evaluation of the patients.

Evaluation

All tests were performed in the experimental and placebo groups at the baseline, 1 and 3 months after the start of the study medication in a double-blinded manner.

OSDI was used for the evaluation of the subjective patient symptoms (17). The patients answered the 12 items on the OSDI questionnaire that were graded on a scale of 0 to 4 (0: none of the time, 1: some of the time, 2: half of the time, 3: most of the time, 4: all of the time). The OSDI was calculated by $([\text{sum of scores for all questions answered}] \times 100) / ([\text{total number of questions answered}] \times 5)$. The ophthalmologic examination included, in the following order: corneal fluorescein staining, a non-anesthetic Schirmer test and impression cytology. The corneal staining was examined with a slit lamp biomicroscope in cobalt blue light 3 min after fluorescein instillation. Punctate staining was recorded with a standardized grading system of 0 to 9. The corneal and conjunctival surface was divided into nine areas, and the numbers from each of the nine stained divisions were added (20). For the Schirmer (I) test, Schirmer strip was inserted over the lower lid margin into the cul-de-sac in the temporal one-third of the lid without topical anesthesia. The strip is removed at 5 min and the extent of wetting of the strip was measured. Conjunctival impression cytology was performed as follows (22). After topical anesthesia with Alcaine[®] (0.5% proparacaine hydrochloride, Alcon-Couvreur, Puurs, Belgium), MF Millipore membrane filters with a pore size of 0.22 μm were applied onto the temporal bulbar conjunctiva. The membrane filter was cut into 5 \times 5 mm asymmetrical pieces with a pointed tip in one corner to guarantee the correct staining surface. With an ophthalmodynamometer, a gentle pressure of 60 g was applied onto the micropore filter membrane for 5 sec. Then, the membrane was fixed in 95% ethanol, stained with periodic acid-Schiff, dehydrated in ethanol (70, 80, 90, 100% in sequence), and next with xylene, and, finally coverslipped. Goblet cells were counted in 10 consecutive high-power fields (HPFs, \times 400). Three observers, who were blinded to the patient treatment, performed all pre- and post-treatment evaluations. Each observer separately examined each test (fluorescein staining, Schirmer test and impression cytology). Another observer who was not involved in any other examination counted the goblet cells.

Statistics

Within each group, the changes in tear secretion, corneal staining, OSDI, and goblet cell counts before and after treatment were compared using a Wilcoxon signed-rank test. To compare the outcome between the groups, we used the Mann-Whitney U test with ratios of the values at 1 and 3 months compared to the values at baseline for minimizing the statistical error caused by individual variation. To know the correlation and difference between right and left eye within each

group, Spearman's rank correlation coefficient and Mann-Whitney U test were used. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

Forty-one patients were initially enrolled and randomly assigned to the medication or placebo group. Fourteen patients were excluded from the analysis because they did not complete the 3-month follow-up. The main reasons for the drop-off were mild gastrointestinal symptoms and poor compliance. Among the 27 patients who completed the trial, 30 eyes of 15 patients were in the oral uridine group and 24 eyes of 12 patients were in the placebo group. There were 3 men and 24 women, with a mean (\pm SD) age of 55 (\pm 15.22) yr (range, 13-76). All patients in both groups showed a moderate to severe dry eye and their previously prescribed medications included preservative-free artificial tear, topical 0.05% cyclosporine (Restasis[®], Allergan Inc., Irvine, CA, U.S.A.),

autologous serum, and teramycin oint. They used no systemic medication for their dry eye.

Corneal and conjunctival fluorescein staining

For the oral uridine treatment group, the post-treatment corneal fluorescein staining scores, after 1 and 3 months of medication, were significantly lower than those obtained at the baseline ($P=0.004$ and $P<0.001$ for 1 and 3-months of medication, Wilcoxon signed rank test). This reduction was significantly lower in the placebo group ($P=0.070$ and 0.117 for 1 and 3-months of medication, Wilcoxon signed rank test) (Fig. 1A). The fluorescein staining score was markedly decreased in the oral uridine group compared to the placebo group at 3-months after treatment ($P=0.032$, Mann-Whitney U test) (Fig. 2A). There was a moderate correlation (oral uridine group, $\rho=0.449$ ($P=0.093$) and 0.607 ($P=0.016$) for 1 and 3-months of medication; placebo group, $\rho=0.751$ ($P=0.008$) and 0.653 ($P=0.029$) for 1 and 3-months of medication) and no significant difference between right and left eye within each both group (oral uridine group, $P=0.642$ and

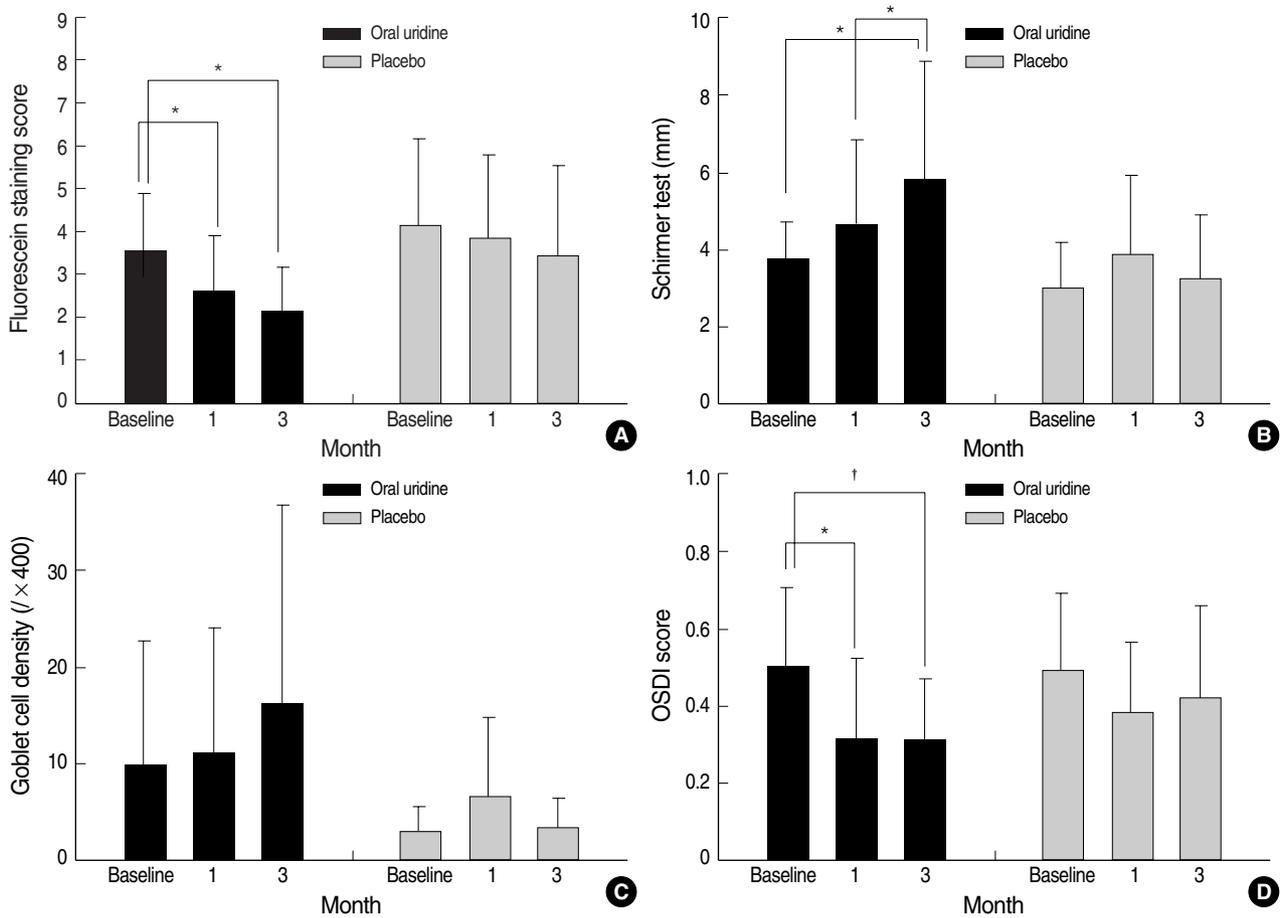


Fig. 1. Change within each group of oral uridine and placebo in conjunctival and corneal fluorescein staining (A), aqueous tear production (B), conjunctival goblet cell density (C) and Ocular Surface Disease Index (OSDI) (D). Data are expressed as mean \pm SD. * $P < 0.01$; † $P=0.044$ (Wilcoxon signed rank test).

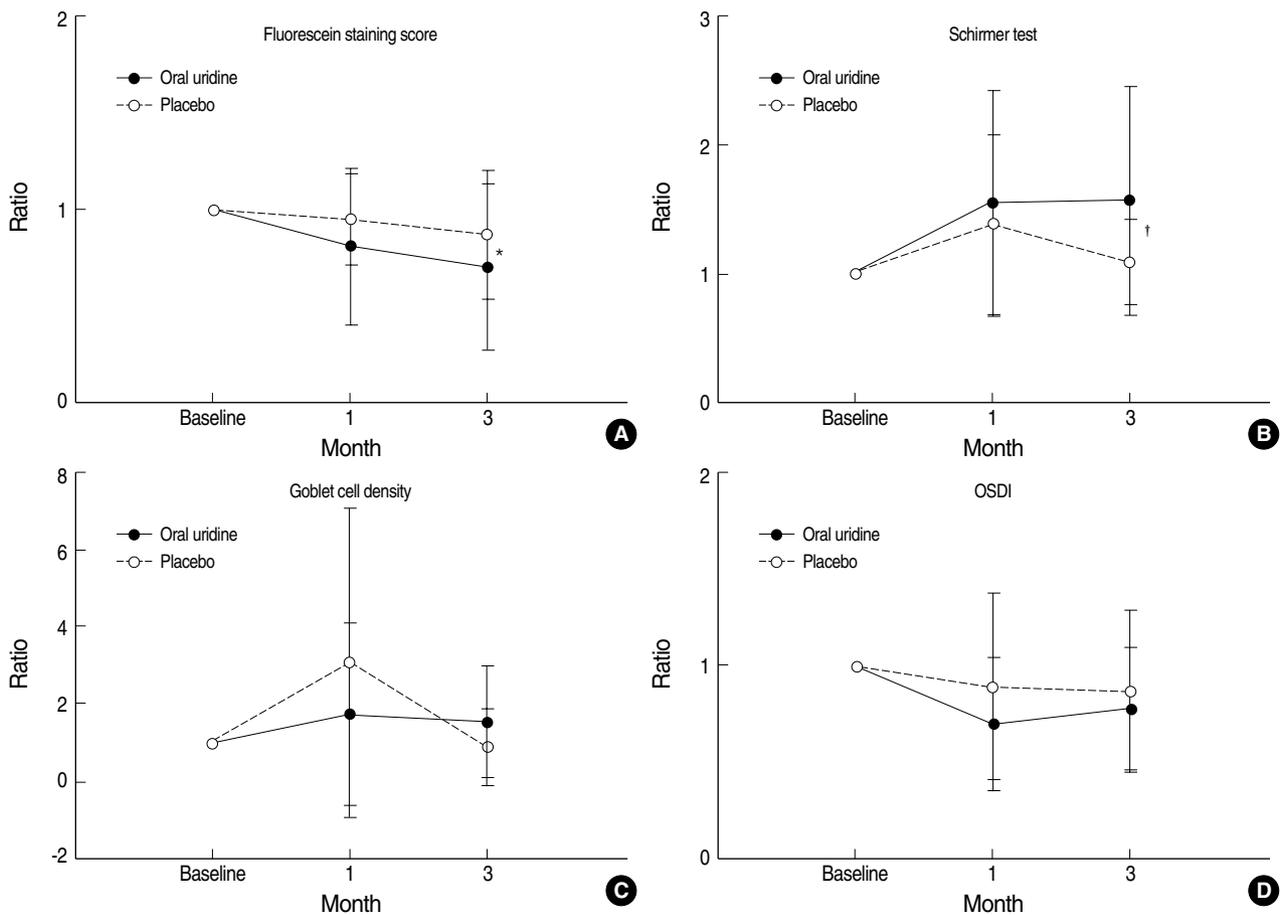


Fig. 2. Change of ratio of the values at 1 and 3 months of treatment to the values at baseline of treatment. This is for comparison of efficacy between two groups in conjunctival and corneal fluorescein staining (A), aqueous tear production (B), conjunctival goblet cell density (C) and Ocular Surface Disease Index (OSDI) (D). Data are expressed as mean \pm SD. * $P=0.032$; † $P=0.030$ (Mann-Whitney U test).

0.439 for 1 and 3-months of medication; placebo group, $P=0.946$ and 0.606 for 1 and 3-months of medication).

Aqueous tear production

The Schirmer wetting score for the oral uridine group was significantly increased ($P=0.001$, Wilcoxon signed rank test), compared with that for the placebo group ($P=0.334$, Wilcoxon signed rank test) at 3-months after treatment (Fig. 1B). The difference between the inter-group ratios between the treatment and control group at 3-months after treatment was also significant ($P=0.030$, Mann-Whitney U test) (Fig. 2B). There was a moderate correlation between right and left eye in oral uridine group ($\rho=0.904$ [$P<0.001$]) and 0.563 ($P=0.023$) for 1 and 3-months of medication) but not in placebo group ($\rho=0.362$ [$P=0.274$] and 0.278 [$P=0.409$]) for 1 and 3-months of medication. There was no significant difference between right and left eye within each both group (oral uridine group, $P=0.776$ and 0.821 for 1 and 3-months of medication; placebo group, $P=0.365$ and 0.478 for 1 and 3-months of medication).

Conjunctival goblet cell density

The goblet cell density, for the oral uridine treatment group, at 1 and 3-months increased; however, the differences were not significant at 1 month ($P=0.660$, Wilcoxon signed rank test) and showed only marginal significance at 3-months ($P=0.053$, Wilcoxon signed rank test) (Fig. 1C). The difference between the oral uridine and placebo group, for conjunctival goblet cell density, at 1 and 3-months after treatment was not significant ($P=0.802$ and 0.201 for 1 and 3 months of medication, Mann-Whitney U test) (Fig. 2C).

Ocular surface disease index

The OSDI was significantly decreased in the oral uridine group at 1 and 3-months ($P=0.004$ and $P=0.044$ for 1 and 3-months of medication, Wilcoxon signed rank test) (Fig. 1D). The change of OSDI in control group after placebo-treatment looked like improved, but it was not significant. However, the difference in the ratios between the two groups, up to 3-months, was not significant ($P=0.506$ and 0.838 for 1 and

3-months of medication, Mann-Whitney U test) (Fig. 2D).

Side effects of the drug

The most common treatment-related adverse events were gastrointestinal symptoms. Six (four in the oral uridine group, and two in the placebo group) of 14 patients were dropped out from the trial because they complained of mild gastrointestinal symptoms such as dyspepsia (n=3), indigestion (n=1) and abdominal discomfort (n=2). After treatment discontinued, the symptoms resolved. No treatment-related serious adverse events occurred during the study.

DISCUSSION

The objective of the present study was to evaluate the efficacy of oral uridine for treatment of the ocular surface in dry eye patients. The results of this study showed that oral uridine treatment had a greater effect on improving the tear production and reducing corneal fluorescein staining than did the placebo.

The tear production for the oral uridine group was significantly increased, compared with that for the placebo group at 3-months after treatment. This result of the present study correspond with the previous studies which P2Y receptor agonists such as UTP stimulate active Cl-transport across the excised rabbit conjunctival tissue *in vitro* (24) and increase tear production in rabbits (25).

The finding of a decrease of corneal staining after the administration of oral uridine was consistent with the results of our previous study. Previously, we demonstrated that the topical application of uridine enhanced healing of a corneal epithelial defect in a rabbit dry eye model. These findings were thought to be associated with the increased biosynthesis of hyaluronate and glycosaminoglycan, or the reduction of MMP-9 by uridine as described in our previous study (11).

The effect of oral uridine, on the goblet cell counts, appears to be different from our earlier study showing a definite increase in the goblet cells with topical uridine. Some possible explanations for these differences are the following. The previous study used rabbit eyes with artificially induced dry eye. The rabbit is well known for proliferation of epithelial (21) or endothelial cells (22, 23) after damage. The goblet cells of rabbits are more rapidly restored after an insult than they are in humans. Another consideration is the difference of severity in dry eye between humans and the rabbits. A dried ocular surface in the rabbits was established by a transient decrease of tear secretion after lacrimal inflammation for 10 days. However, many different factors are involved in the development of dry eye in humans. In addition, the presence of dry eye in the patients was much longer than in the rabbits. Our results showed that the goblet cells tended to gradually increase until 3-months after the start of treatment with

a marginal significance when compared to the placebo group.

We found that oral uridine treatment was associated with a reduced OSDI. Since the subjective symptoms of dysfunctional tear syndrome are used as diagnostic criteria, our results suggest that this treatment may be helpful for patients with dysfunctional tear syndrome even without signs of damage to the ocular surface. Treatment with oral uridine significantly improved the subjective symptoms of dry eye. However, placebo group also showed some reduction of OSDI which turned out to be insignificant and was assumed to be caused by "placebo-effect". It might affect the result which presented no significant difference between those groups. To clarify that issue, large number of prospective study is pending.

Diquafosol tetrasodium (INS365) (8), has also demonstrated improvement in tear production in randomized, controlled ongoing clinical trials. Diquafosol, a P2Y₂ receptor agonist, which is an analogue of the endogenous compound UTP, is thought to contribute to the restoration of the normal integrity of the ocular surface by promoting fluid and mucin secretion, regardless of lacrimal gland function (24, 25). Uridine is transformed to UDP or UTP by uridine kinase *in vivo*. In a similar manner to diquafosol, the results of our study showed that uridine has a similar effect on the ocular surface, as does the P2Y₂ receptor agonist. Furthermore, uridine administration appears to induce cross-talk for the synthesis of hyaluronate, as discussed in our previous study (11). Several other studies showed elevated UDP-glucose with the synthesis of hyaluronic acid (12, 13). Therefore, oral uridine may have a dual role, the increase of hyaluronic acid synthesis and as a P2Y₂ receptor agonist, possibly resulting in restoration of healthy ocular surface including increase of goblet cells.

Meanwhile, several studies showed that repeated exposure with purinergic agonist such as UTP causes receptor desensitization *in vitro* (26-28). Therefore, adequate frequency and dosage of UTP derivatives to avoid desensitization seems to be crucial for being effective on ocular surface in dry eye patients. Although we found application of uridine 3 times a day (300 mg/day) for 3 months is likely to be working on ocular surface, we do not know the optimal frequency and dosage of uridine to maximize the effect and whether long term application of daily dosage may induce desensitization or not. Further study would be warranted to evaluate desensitization.

The effects of oral uridine seemed to be more prominent at 3-months of treatment than at 1-month. Although uridine has been used as a therapeutic agent in the treatment of several other medical disorders (29-31), we do not know the precise bioavailability of the uridine which we provided to the patients. Regarding the clinical use of oral uridine has been limited by its poor oral bioavailability (7-8%) and short plasma elimination half-life (32, 33), further investigation of its bioavailability is needed to improve these limitations.

In order to determine the exact mechanism of action of

oral uridine on the ocular surface and proper dosage for dry eye, another study on the relationship between the expression of hyaluronan synthase (HAS) or the P2Y₂ receptor with administration of oral uridine, in an in vivo model, is currently under way.

For the safety of oral uridine, prior uridine studies (32-34) using high doses of oral uridine or uridine prodrugs have shown the drug to be well tolerated in humans; osmotic diarrhea sometimes has been reported as dose-limiting and uridine is now commercially available as sugarcane extracts in the world. In our study there were also several patients having mild gastrointestinal symptom after medication. Of them, four patients belong to oral uridine group and two belong to placebo group. In fact, the uridine which we used was not a pure uridine but a combination with other components including L-glutamine, lactose and crystalline cellulose. Therefore, gastrointestinal symptom might be possibly caused by other components as well as uridine. These side effects were mild and resolved after discontinuation of the medication.

This study has several limitations. First, the small number of patients was enrolled. A larger randomized clinical trial is required to confirm the safety and efficacy of oral uridine. Second, the tear secretion was measured by the Schirmer test, which has been criticized for its variability and low sensitivity (18). However, it is still a useful way of measuring the aqueous tear production; it is a simple test, with low cost and wide availability, and it has been used in many clinical studies on dry eye. Like other studies, Schirmer I test (without topical anesthesia) was also used in our study. Schirmer I test better reflects the reflex tearing and ocular surface abnormalities in patients with dry eye as compared to the schirmer test with anesthesia because the reflex tearing is important for the maintenance of the ocular surface (35). Third, the design of the study was, at some point, limited because the patients had been allowed to keep their most previously prescribed topical eyedrops except the hyaluronic acid eye drops. Because the patients suffered from intractable dry eyes even though all those medication, ethically we could not stop giving them those drops. We admit those differences of topical medication between treated and control groups may have a confounding effect on analyzing the influence of oral uridine. However, we did not directly compare treated group with control group. Considering that the changes of post-treated eye from the pre-treated were indirectly compared using the ratio in each group, we still believe this study shows clinical relevance in some extent.

Despite several limitations, to the best of our knowledge, this is the first study showing that oral uridine may be helpful for patients with moderate-to-severe dry eye syndrome at least for 3 months.

The results of this study showed that the administration of oral uridine was likely to be safe and effective; it increased tear secretion, decreased fluorescein staining of the damaged cornea and improved patient subjective symptoms.

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