patients with severe OSDs. Based on these promising results, novel methods have been developed for the cultivation of allogeneic corneal[7,8,21] or autologous oral mucosal[22–25] epithelial cells on a denuded amniotic membrane. Immunologic rejection and increased risk of infection or systemic adverse effects associated with the long-term immunosuppressive therapy accompanying allograft transplantation[6] encouraged changing to autologous cultivated oral mucosal epithelial transplantation (COMET) in patients with severe OSDs in 2002.[10,11,23,26]

To clarify the effectiveness, disease-specific outcomes, and safety of COMET, all of the clinical data from all 72 patients that the authors treated with COMET since 2002 were analyzed. The objective of this present study was to summarize the long-term clinical outcomes of 40 of those 72 patients who underwent COMET with the primary objective of visual improvement between June 2002 and December 2008.

Patients and Methods

Patients

Autologous COMET was performed on consecutive patients who were diagnosed with total LSCD based on the complete disappearance of the palisades of Vogt and 360° of conjunctivalization.[1] The COMET treatment protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine, Kyoto, Japan, in 2002. The final decision to perform COMET was made by the university's team of corneal specialists. Before the surgery, written informed consent was obtained from all patients in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. The current retrospective study used an itemized data collection form, and the medical records of all patients who underwent COMET between June 2002 and December 2008 were examined retrospectively. This retrospective study protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine in 2009. In this study, 40 of the 72 patients who underwent COMET were analyzed with the primary objective of visual improvement.

Cell Culture

All of the COMET sheets were prepared at the good manufacturing practices–graded Cell Processing Center at Kyoto Prefectural University of Medicine as previously described.[23,26] Autologous oral mucosal epithelial cells were obtained from a 6-mm–diameter biopsy specimen obtained from each patient's buccal mucosa, and the cells then were cultured on an amniotic membrane spread on the bottom of a culture insert and were cocultured with mitomycin C-inactivated 3T3 fibroblasts (NIH-3T34; RIKEN Cell Bank, Tsukuba, Japan). The cultured cells were submerged in medium for approximately 1 week and then were exposed to air by lowering the medium level (airlifting) for 1 to 2 days. All amniotic membrane was obtained from caesarean sections according to the preparation method described previously.[23] Although fetal bovine serum initially was used as a culture medium, autologous serum was used in later cultures to reduce the risk of transmitting nonhuman pathogens.[26]

Transplantation and Postoperative Management

The surgical procedure (see the Supplemental Video, available at http://aaojournal.org) and postoperative management have been described previously.[24,25] In patients with severe symblepharon or a large area of bare sclera exposed during surgery, amniotic membrane was transplanted onto the bare sclera to reconstruct conjunctival fornices.[18] In patients with a cataract, phacoemulsification and aspiration plus intraocular lens implantation were performed simultaneously with COMET. No penetrating keratoplasty or deep lamellar keratoplasty was performed simultaneously with COMET. For patients with severe corneal stromal opacity, a 2-step surgical approach was planned, with the first step being COMET and the second step being either penetrating or deep lamellar keratoplasty.[25]

Systemic corticosteroid (betamethasone, 1 mg/day) and cyclosporine (2 to 3 mg/kg daily) were administered to prevent postoperative inflammation and immunologic response and then were tapered, depending on the clinical findings. Dexamethasone (0.1%) and antibiotic eye drops were instilled 4 times daily. Dry-eye patients were administered artificial tears. A therapeutic soft contact lens was used for at least 1 month to protect transplanted epithelium from mechanical ablation.

Postoperative Follow-up and Outcomes

Best-corrected visual acuity (BCVA) was converted to the logarithm of the minimum angle of resolution (logMAR). Ocular surface conditions including corneal appearance (epithelial defects, clinical conjunctivalization, neovascularization, opacification, keratinization, and symblepharon) were graded by at least 2 ophthalmologists (C.S., T.I., and T.N.) on a scale from 0 to 3 according to their severity, in accordance with a previously reported grading system.[27] Severe OSDs are characterized by an associated loss of conjunctival stem cells, and the severity of conjunctival involvement affects the visual prognosis. Therefore, findings on upper and lower fornix shortening were added to evaluate the grade of conjunctival appearance. Fornix shortening was graded from 0 to 3 based on the following clinical features: normal depth (grade 0), shortened by less than one quarter (grade 1), shortened by one quarter to one half (grade 2), and shortened by more than one half (grade 3). Upper and lower fornix shortenings were graded separately. The sum of each grading score was defined as the ocular surface grading score (maximum, 24).

Each patients logMAR BCVA, ocular surface grading score, and data on adverse events related to COMET or postoperative management were collected from the medical records at these specific time points: before surgery; at the 4th, 12th, and 24th postoperative weeks; and at the last follow-up examination. The primary outcome was the change in logMAR BCVA at the 24th postoperative week. Because other ocular diseases can affect this visual outcome, a secondary outcome, the ocular surface grading score, also was defined.

Statistical Analysis

The change in BCVA and ocular surface grading score from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except for other diseases. Multivariate stepwise logistic regression analysis was used to determine the factors influencing visual improvement.

This study defined the critical visual improvement rate as the proportion of patients in whom BCVA at the 24th postoperative week had improved to at least 0.01, as a percentage of the patients with a BCVA of less than 0.01 at baseline. Patients with a visual acuity of 0.01 or more can read and walk using vision aids. Thus, an improvement to at least 0.01 indicates a capacity for independence in daily life. If data were missing from the 24th postoperative week, data from follow-up at the last visit were substituted.

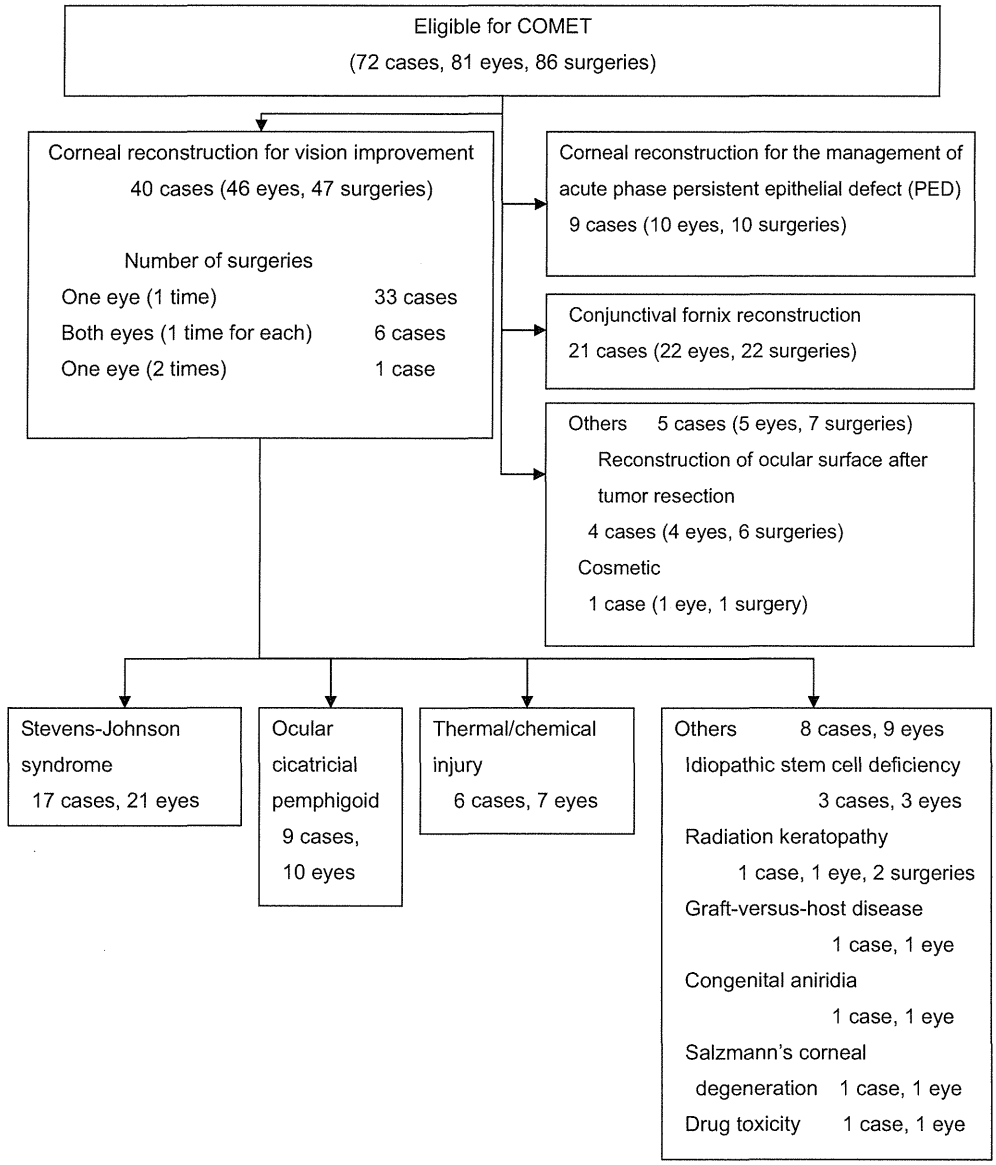

Figure 1. Diagram showing flow of study. Seventy-two patients (81 eyes) underwent cultivated oral mucosal epithelial sheet transplantation (COMET) between June 2002 and December 2008, and 40 patients (46 eyes) were analyzed for visual improvement in this study. Both corneal reconstruction and conjunctival fornix reconstruction were carried out in 3 cases, in the same eye in 1 case, and counted separately.

All statistical analyses were conducted at the Translational Research Informatics Center (Kobe, Japan) with the use of SAS software, version 9.1 (SAS Inc, Cary, NC) or JMP software, version 8.2 (SAS Inc). *P* values of less than 0.05 were considered statistically significant.

Results

Patient Characteristics

Between 2002 and 2008, 47 COMETs (46 eyes in 40 patients) were performed on 21 eyes with SJS, 10 eyes with OCP, 7 eyes with thermal or chemical injury, and 9 eyes with other causes of LSCD (Fig 1). Although 23 eyes (48.9%) previously had been treated with ocular surgery, all of these previous treatments had failed and recurrence of fibrovascular ingrowth on the cornea was observed. Of the 47 surgeries performed, symblepharon and keratinization of the cornea were present in 37 eyes (78.7%) and 10 eyes (21.3%), respectively, thus indicating that most of the eyes were inflicted with end-stage severe OSDs (Table 1).

Outcomes of Cultivated Oral Mucosal Epithelial Sheet Transplantation

Cultivated autologous oral mucosal epithelial sheets were generated successfully from all patients. In all patients, COMET was performed successfully and no epithelial damage was observed during surgery. Cultivated oral mucosal epithelial sheet transplantation was combined with amniotic membrane transplantation in 34 (72%) of the 47 surgeries and with cataract surgery in 11 eyes (23%; Table 2, available at http://aaojournal.org). In 10 patients

Table 1. Baseline Characteristics in Patients Who Underwent Autologous Cultivated Oral Mucosal Epithelial Transplantation

	Total	Stevens-Johnson Syndrome	Ocular Pemphigoid	Thermal/Chemical Injury	Others
No. of COMETs	47	21	10	7	9
Age (yrs)					
Median	57.0	43.0	73.5	50.0	34.0
Range	9–86	14–71	62–86	27–79	9–75
Duration of illness (yrs)					
Median	12.3	17.9	3.5	6.0	5.08
Range	0.3–40.0	3.0–38.0	0.3–15.0	0.5–24.0	0.5–40.0
Prior ocular surgery (%)	23 (48.9)	9 (42.9)	4 (40.0)	3 (42.9)	7 (77.8)
Planned 2-step operations (%)	10 (21.3)	2 (9.5)	0 (0)	6 (85.7)	2 (22.2)
Symblepharon (%)	37 (78.7)	18 (85.7)	10 (100.0)	6 (85.7)	3 (33.3)
Keratinization (%)	10 (21.3)	8 (38.1)	1 (10.0)	0 (0)	1 (11.1)
Preoperative visual acuity*					
Median	2.40	2.4	2.70	2.70	2.40
Range	1.11–3.00	1.40–3.00	1.52–2.70	1.22–2.70	1.10–2.70
Preoperative ocular surface grading score					
Median	14.0	15.0	17.0	13.0	8.0
Range	5.0–21.0	8.0–21.0	10.0–21.0	7.0–17.0	5.0–19.0

COMET = autologous cultivated oral mucosal epithelial transplantation.
*Logarithm of the minimum angle of resolution units.

with severe corneal stromal opacity, a 2-step surgical approach was planned, with COMET followed by penetrating keratoplasty or deep lamellar keratoplasty.[25] Three patients underwent the second surgery before the 24th postoperative week and 5 patients underwent the surgery after the 24th week, but 2 patients did not undergo the second surgery during the study period.

The median preoperative logMAR BCVA was 2.40, and in 31 of the eyes (66%), visual acuity was poorer than 20/2000 (<0.01, logMAR >2). The median preoperative ocular surface grading score was 18.0 (range, 5 to 21). The median patient follow-up period with observation of the primary outcome was 28.7 months after transplantation (range, 6.2 to 85.6 months). Because of heterogeneous etiologic mechanisms, the outcomes in each category are described separately.

Disease-Specific Outcomes

Stevens-Johnson Syndrome. Seventeen patients with SJS underwent COMET (Table 2, available at http://aaojournal.org). The BCVA improved significantly at 4, 12, and 24 weeks after surgery ($P = 0.0005$, $P = 0.0010$, and $P = 0.0117$, respectively; Fig 2A). The ocular surface grading score also improved significantly at 4, 12, and 24 weeks after surgery ($P<0.0001$ for each time point; Fig 2B).

Ocular Cicatricial Pemphigoid. Nine patients (10 eyes) with OCP underwent COMET (Table 1). All 9 patients were older than 60 years, older than many of the patients in this study with other diseases (Table 2, available at http://aaojournal.org). The BCVA was improved significantly at the 4th postoperative week ($P = 0.0156$), but this improvement later disappeared (Fig 2A). In contrast, improvement of the ocular surface grading score was sustained throughout the follow-up period ($P = 0.0020$, $P = 0.0020$, and $P = 0.0078$, respectively; Fig 2B).

Thermal or Chemical Injury. Seven patients (7 eyes) with thermal or chemical injury underwent COMET. Their BCVA did not change until the 24th postoperative week; however, the ocular surface grading score in all 7 patients improved significantly ($P = 0.0156$ for each visit; Fig 2A, B). Although penetrating keratoplasty or deep lamellar keratoplasty surgery was planned for 6 of these 7 patients, only 2 patients underwent this second surgery before the 24th postoperative week visit. Both the BCVA and ocular surface score improved in all 7 patients after the planned surgeries were performed.

Others. Eight other patients underwent COMET: 3 with idiopathic stem cell deficiency, 1 with radiation keratopathy, 1 with graft-versus-host disease, 1 with congenital aniridia, 1 with Salzmanns corneal degeneration, and 1 with drug-toxicity–induced LSCD. In 6 of these 8 patients, BCVA was improved significantly; however, no improvement was seen in 2 of these patients (Table 2, available at http://aaojournal.org; Fig 2A). The 2 patients with no improvement had severe dryness on the ocular surface and had the highest ocular surface grading score in this group. In addition, severe lagophthalmos was present in the 1 patient with radiation keratopathy because of severe lid scarring after irradiation for retinoblastoma. One other patient with graft-versus-host disease had longstanding inflammation on the ocular surface. In both of these 2 cases, keratinization and symblepharon progressed gradually after COMET. Six patients who demonstrated improvement had a low preoperative ocular surface grading score, yet this score was improved considerably in all patients at the 24th postoperative week (Table 2; Fig 2B).

Critical Visual Improvement Rate

The critical visual improvement rate for SJS, OCP, and thermal or chemical injury was 50.0% (7/14), 42.9% (3/7), and 20.0% (1/5), respectively, although the second planned surgery[25] (penetrating or deep lamellar keratoplasty) had yet to be carried out at the 24th postoperative week in 7 of 10 eyes. The clinical observations on both preoperative and postoperative anterior segment slit-lamp photographs are shown in Figure 3 (available at http://aaojournal.org). All patients demonstrated an improvement in their BVCA to 0.01 or more, from a baseline condition of vision loss.

Factors Influencing Visual Improvement

Multivariate stepwise logistic regression analysis was used to estimate the factors influencing postoperative visual acuity after COMET, and the following factors were chosen as variables:

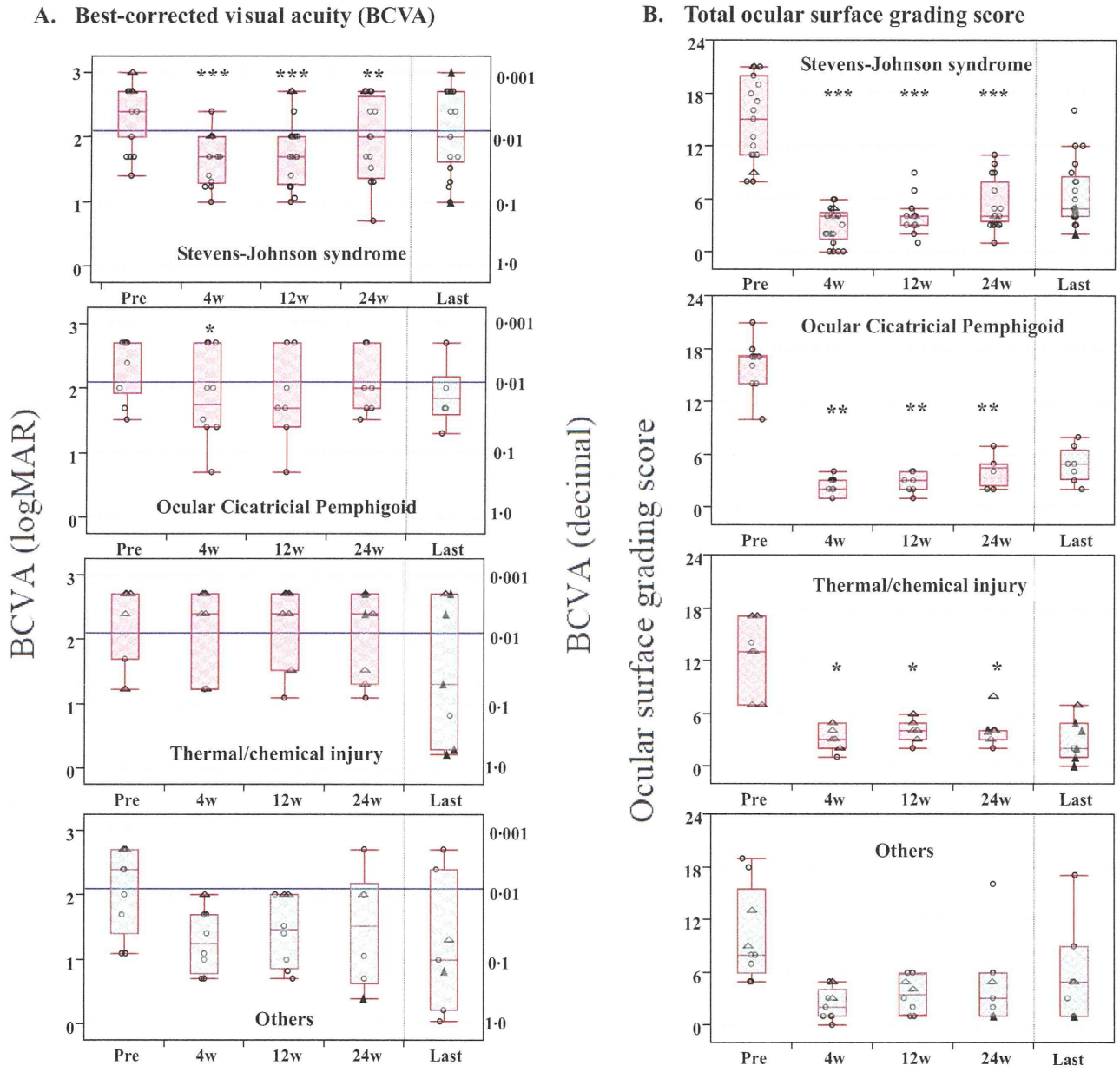

Figure 2. Graphs showing preoperative (Pre) and postoperative clinical outcomes. **A**, Best-corrected visual acuity (BCVA). The BCVA values for each patient are shown grouped according to the cause of corneal dysfunction: Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), thermal or chemical injury, and others. The change in BCVA from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except others. Open circles represent cases treated with autologous cultivated oral mucosal epithelial transplantation (COMET) only. Triangles represent cases treated with a planned 2-step surgical combination of COMET followed by penetrating keratoplasty (PK) or deep lamellar keratoplasty (DLKP). Open triangles are before the second operation, and closed triangles are after the second operation. The horizontal line within each box represents the median value, the bottom and top lines of the box represent the 25th and 75th percentiles, respectively, and the horizontal lines below and above the box represent the lowest and highest values, respectively (or are located 1.5 times the interquartile range away from the box). *P<0.05, **P<0.01, ***P<0.001 (2-sided). **B**, Total ocular surface grading score. Ocular surface grading scores for each patient were calculated and are shown according to each cause of corneal dysfunction: SJS, OCP, thermal or chemical injury, and others. Scores for 8 components of the ocular surface were calculated by the grading system. The total scores before surgery and at the 4th, 12th, and 24th postoperative weeks and at last follow-up examination were calculated. Open circles represent patients treated with COMET only. Triangles represent patients treated with a planned 2-step surgical combination of COMET followed by PK or DLKP. Open triangles are before the second operation, and closed triangles are after the second operation. The change in ocular surface grading score from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except others. The horizontal line within each box represents the median value, the bottom and top lines of the box represent the 24th and 75th percentiles, respectively, and the horizontal lines below and above the box represent the lowest and highest values, respectively (or are located 1.5 times the interquartile range away from the box). *P<0.05, **P<0.01, ***P<0.001 (2-sided). w = weeks.

Table 3. Summary of Adverse Events in Patients Who Underwent Autologous Cultivated Oral Mucosal Epithelial Transplantation

Event	Total	Disease Category			
		Stevens-Johnson Syndrome	*Ocular Cicatricial Pemphigoid*	*Thermal or Chemical Injury*	*Others*
Hepatic dysfunction	1	1			
Drug-induced allergy	1				1
Persistent epithelial defect	16	10	3	2	1
Corneal stromal melting after the epithelial defect	2		1		1
Ocular infection (keratitis, endophthalmitis)	2	2			
Infiltration	3	2	1		
Elevation of IOP resulting from steroid use	4		1	2	1

IOP = intraocular pressure.
No life-threatening serious adverse events were observed.

disease category, patient age, 2-step surgery, combination with amniotic membrane transplantation, combination with cataract surgery, preoperative logMAR BCVA, and the 8 components of the ocular surface grading system. Corneal neovascularization and symblepharon were found to be correlated significantly with logMAR improvement at the 24th postoperative week ($P = 0.0023$ and $P = 0.0173$, respectively). Visual prognosis was better in the eyes with slight symblepharon than in the eyes with severe symblepharon. In contrast, it was better in the eyes with severe neovascularization than in the eyes with slight neovascularization.

Adverse Events

A summary of the adverse events in the 40 patients who underwent COMET is shown in Table 3. No life-threatening serious adverse events were observed in any of the transplantations. Systemically, moderate liver dysfunction occurred in 1 patient (2.5%; 95% confidential interval [CI], 0.1 to 13.2), but liver function normalized after the discontinuationof systemic drugs.

Postoperative persistent epithelial defects occurred in the eyes of 16 (40.0%) of the 40 patients (95% CI, 24.9 to 56.7), and rather frequently in the SJS eyes (60.0% of SJS patients). Corneal stromal melting after the epithelial defect occurred in 2 patients (5.0%; 95% CI, 0.6 to 16.9), but neither eye became perforated. All of these patients were treated successfully. Slight to moderate corneal infection occurred in 2 patients (5.0%; 95% CI, 0.6 to 16.9); however, both patients healed without scarring. A suspected infection with cell infiltration on the cornea[28] occurred in 3 patients, yet in each patient, it healed within 1 week after receiving a topical instillation of antibiotics. Although a slight elevation of intraocular pressure resulting from steroid use was seen in 4 patients (10.0%; 95% CI, 2.8 to 23.7), this returned to the normal range after reduction of the steroid dose. None of the patients required glaucoma surgery.

Discussion

Severe OSD has proven to be one of the most difficult disorders to treat, and for many patients, vision loss is the end result.[29,31] Keratoprosthesis surgery is one possible way to obtain visual improvement in end-stage severe OSDs; however, serious complications such as endophthalmitis, glaucoma, and tissue melting can arise, especially in SJS or OCP, and can lead to permanent vision loss.[32,33]

At the beginning of 2002, the authors performed ocular surface reconstruction using tissue-engineered autologous oral mucosal epithelial sheets for the first time.[23] In a report of the initial results from the first 12 cases, the successful long-term engraftment of cultivated oral mucosal cells and their transparency was confirmed.[24] Since then, COMET has been used to treat OSD patients, with careful consideration of the surgical indications.[24–26,34] The authors performed 86 COMET operations between 2002 and the end of 2008 for visual improvement, epithelialization of persistent epithelial defects, or conjunctival reconstruction (Fig 1).

In this study, the clinical efficacy and safety of 47 COMETs were evaluated for visual improvement. In 23 eyes (48.9%), previous ocular surgery such as corneal transplantation or amniotic membrane transplantation already had been carried out unsuccessfully at other hospitals. Symblepharon was involved in 37 eyes (78.7%) and keratinization was involved in 10 eyes (21.3%). Symblepharon indicates conjunctival involvement, and pathologic keratinization means that the eye is at the end stage of a severe OSD with chronic inflammation.[3,35] Most of these eyes had severe tear deficiency, which is an important prognostic parameter for surgical outcome.[36] Although such eyes commonly are considered to have contraindications for ocular surface reconstruction, COMET offered substantial visual improvement even for patients with such advanced disease.

In more than half of the eyes, preoperative visual acuity was limited to counting fingers or hand movements. It is striking that such patients were able to come to the hospital without assistance during the 24 weeks after undergoing COMET. For this reason, critical visual improvement rate is proposed as a clear end point for measuring surgical outcome. Considering that most of the eyes in this study were at the end stage of a severe OSD, these results are very favorable and encouraging.

In this study, the preoperative ocular surface grading score was higher (more diseased) in patients with SJS and OCP than in those with thermal or chemical injuries or other

diseases. It should be noted that visual improvement was statistically significant in SJS. In contrast, visual acuity was not improved at the 24th postoperative week in patients with thermal or chemical injury, despite the improvement in total ocular surface grading score. The corneal stroma was damaged severely in most cases of thermal or chemical injury, and such patients obtained visual improvement after undergoing the planned second surgery with penetrating keratoplasty or deep lamellar keratoplasty. In general, the prognosis of penetrating or deep lamellar keratoplasty alone for severe OSDs is very poor.[2] However, the findings of this study show that patients with severe OSDs with corneal stromal opacity can obtain visual improvement after undergoing the surgical combination of COMET and penetrating or deep lamellar keratoplasty.

Best-corrected visual acuity was not improved at the 24th postoperative week in patients with OCP, despite significant improvement of the ocular surface grading score. Because OCP is a progressive autoimmune disease, pathologic keratinization or thickening of the epithelium occurred readily after COMET, thus disrupting visual acuity.

No serious systemic complications occurred in any of the patients. The incidence of postoperative persistent epithelial defects was relatively high, yet still similar to or lower than that reported with other therapies.[6,36–38] Considering that corneal perforation is a common complication after corneal reconstruction in severe OSDs,[38–40] it is noteworthy that no perforation occurred and that none of the eyes demonstrated vision loss after COMET. Ocular surface reconstruction with a combination of COMET and amniotic membrane transplantation was needed to achieve the total replacement of cicatrized tissue. Because cultured epithelial cells on amniotic membrane attach to a basement membrane with hemidesmosomes,[22] these cells can avoid being dropped off and actually survive, regardless of an unstable tear film and the mechanical trauma of blinking. When used as the substrate for oral mucosal cells, amniotic membrane may play a role in protecting the cornea from melting.

Multivariate stepwise logistic regression analysis showed that symblepharon and neovascularization are prognostic factors for visual improvement. Although disease-specific outcomes showed different patterns as described above, disease category was not related to visual prognosis. However, the sample size may be too small to perform such subgroup analyses. Multivariate stepwise logistic regression analysis also was performed for all 86 surgeries to determine the factors influencing persistent epithelial defects. Having SJS and a very low tear meniscus were the prognostic factors for persistent epithelial defects ($P = 0.0204$ and $P = 0.0388$, respectively). Thus, it is likely that both the disease category and dryness of the eye influenced the prognosis.

Long-term ocular surface appearance was examined in 17 of the 72 patients with a follow-up of more than 3 years.[34] No further surgery was carried out in these patients. The ocular surface in each case became stable from 6 months after COMET, with a gradual reduction in corneal neovascularization,[34] as others have reported in similar studies.[40] Moreover, postoperative invasion of conjunctival tissue and symblepharon formation was inhibited significantly for more than 3 years.[34] Deep lamellar or penetrating keratoplasty was performed for the patients with corneal stromal opacity after the stabilization of the ocular surface (as the second step of a 2-step surgical combination), in most cases from 24 weeks after COMET.

After COMET, upper or lower eyelid cicatricial entropion with various degrees of tarsal-plate atrophy sometimes was found. In cases with an eyelid abnormality, eyelid surgery was performed to correct entropion, trichiasis, or lagophthalmos. Eyelid condition is an important factor for maintaining ocular surface stability, as well as for avoiding complications such as infection or persistent epithelial defects.

In conclusion, the findings of this retrospective study showed that long-term visual improvement can be obtained in end-stage severe OSDs with complete LSCD and that COMET offered substantial visual improvement even for patients with severe tear deficiency. The findings also showed that patients with corneal blindness resulting from severe OSDs such as SJS benefited from critical improvement of visual acuity.

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Research Article

In Silico Risk Assessment of HLA-A*02:06-Associated Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis Caused by Cold Medicine Ingredients

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe drug hypersensitivities with high mortality. Typical over-the-counter drugs of cold medicines are suggested to be causative. As multiple ingredients are generally contained in cold medicines, it is of particular interest to investigate which ingredients are responsible for SJS/TEN. However, experimental examination of causal relationships between SJS/TEN and a particular drug molecule is not straightforward. Significant association between HLA-A*02:06 and SJS/TEN with severe ocular surface complications has been observed in the Japanese. In the present study, we have undertaken *in silico* docking simulations between various ingredients contained in cold medicines available in Japan and the HLA-A*02:06 molecule. We use the composite risk index (CRI) that is the absolute value of the binding affinity multiplied by the daily dose to assess the potential risk of the adverse reactions. The drugs which have been recognized as causative drugs of SJS/TEN in Japan have revealed relatively high CRI, and the association between SJS/TEN and HLA-A*02:06 has been qualitatively verified. The results have also shown that some drugs whose links to SJS/TEN have not been clinically recognized in Japan show the high CRI and suggested that attention should be paid to their adverse drug reactions.

1. Introduction

Adverse drug reactions (ADRs) are a serious public health problem. Drug hypersensitivity which constitutes a major category of ADRs is typically severe in nature. In particular, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe drug hypersensitivities with high mortality. Various drugs have been pointed out as causes of SJS/TEN. In addition to certain prescription drugs, typical over-the-counter drugs of cold medicines are suggested to be causative [1, 2]. These reactions often result in severe and definitive sequelae such as vision loss in some cases [2]. The reported incidence of ocular complications in SJS/TEN is 50–68% [3].

Considering the fact that cold medicines are the most widely used over-the-counter drugs, it is extremely important to know the potential causative ingredients of SJS/TEN. Although nonsteroidal anti-inflammatory drugs which are major ingredients of cold medicines were suggested as causative drugs [2], no systematic studies about drug hypersensitivities of other ingredients have been undertaken. Since it is possible that certain ingredients might cause more severe

Table 1: Ingredients contained in popular cold medicines available in Japan. Chinese herbs, vitamins, and lysozyme are excluded from the table.

Drug name	Therapeutic category
Acetaminophen	Analgesic; antipyretic
Ambroxol	Expectorant
Bromhexine	Expectorant; mucolytic
Caffeine	CNS stimulant; respiratory stimulant
Carbinoxamine	Antihistaminic
d-Chlorpheniramine	Antihistaminic
Clemastine	Antihistaminic
Dextromethorphan	Antitussive
Dihydrocodeine	Analgesic (narcotic); antitussive
Ethenzamide	Analgesic
Guaiacol	Expectorant
Guaifenesin	Expectorant
Ibuprofen	Anti-inflammatory; analgesic; antipyretic
Isopropamide	Antispasmodic
Loxoprofen (*R*, *S*)	Anti-inflammatory; analgesic
Mequitazine	Antihistaminic
d-Methylephedrine	Analeptic
Noscapine	Antitussive
Pseudoephedrine	Decongestant
Tranexamic acid	Hemostatic

adverse reactions, predicting the potential adverse reactions of other ingredients will be undoubtedly beneficial to avoid the latent ADRs.

Significant associations between certain ADRs and specific alleles of human leukocyte antigen (HLA) have been pointed out [4]. Recently, the detailed molecular mechanism underlying the strong association between HLA-B*57 and the hypersensitivity of reverse-transcriptase inhibitor abacavir has been disclosed [5]. The relevant adverse drug reaction is triggered by the strong binding of abacavir into the antigenic peptide binding groove of the HLA molecule. It has been reported that the frequency of carriers of the HLA-A*02:06 antigen is significantly higher among Japanese patients with severe ocular surface complications than in other populations [6]. It is highly possible that the adverse drug reactions associated with HLA-A*02:06 are also triggered by the direct interactions between the causative drug molecules and the HLA molecule.

However, it is generally intractable to ascertain experimentally the causation between a specific drug and an incidence of a particular adverse drug reaction especially in cases where multiple drugs are administered simultaneously such as cold medicines. *In silico* analysis based on the underlying molecular mechanism of the ADRs can be employed as a powerful alternative method in these situations. In this study, *in silico* analysis using docking simulations between various ingredient molecules in cold medicines and the HLA-A*02:06 molecule has been undertaken in order to predict what ingredient molecules might cause SJS/TEN through the interactions with HLA-A*02:06.

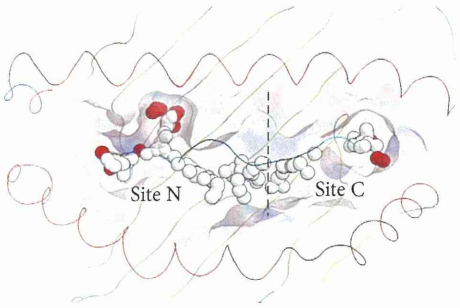


Figure 1: The antigenic-peptide binding groove of HLA-A*02:06 is depicted schematically with the two alpha helices represented by helical lines running horizontally. The white and red alpha spheres calculated in the groove represent hydrophobic and hydrophilic positions, respectively. The binding groove is divided into sites C and N.

2. Methods

We examined all ingredients contained in four popular over-the-counter cold medicines available in Japan, that is, Lulu, Pabron, Contac, and Benza Block. The ingredient molecules used in docking simulations are given in Table 1. As Chinese herbs contained in all of these over-the-counter cold medicines are mixtures of various agents whose molecular structures are not fully identified, they are excluded from the docking simulations. Lysozyme and vitamins are also excluded. A software system molecular operating environment (MOE) [7] was used throughout this study and all the calculations were performed on a DELL PC workstation T7500.

The X-ray structure of the HLA-A*02:06 molecule deposited in the Protein Data Bank [8] (PDB ID: 3OXR) was used for docking simulations. It is highly possible that the molecules shown in Table 1 bind to the antigenic-peptide binding groove located between two α helices as in the case of abacavir. Since the groove is relatively wide, we have specified the possible binding sites by use of the alpha site finder function [9] implemented in MOE. Small spheres named alpha spheres shown in Figure 1 correspond to locations of tight atomic packing at the antigenic-peptide binding groove. A site where the alpha spheres are clustered is designated alpha site which is considered to be the potential binding site of drug molecules. Two alpha sites designated sites N and C were identified in the antigenic-peptide binding groove of the HLA-A*02:06 molecule and these two sites were considered in docking simulations. All docking simulations were undertaken by use of software ASEDock [10]. ASEDock based on unique concept of ASE model and ASE score uses the alpha sites for docking the small molecule. Since ASEDock is free from any bias except for shape, it is one of the very robust docking methods. The binding affinity of a drug molecule to the HLA molecule was judged by

Table 2: Binding affinities of the ingredients of cold medicines to the antigenic-peptide binding groove of HLA-A*02:06. Composite risk index is calculated by multiplying the maximum daily dose and the absolute value of GBVI/WSA_dG.

Drug name	Maximum dose/day (mmol)	GBVI_dG* (site N) (kcal/mol)	GBVI_dG* (site C) (kcal/mol)	Composite risk index (site N)**	Composite risk index (site C)**	Preferential binding site
Ethenzamide	6.356	−4.966	−5.335	31.565	33.914	C
Acetaminophen	5.954	−4.623	−4.608	27.523	27.438	N
Tranexamic acid	4.771	−5.550	−5.613	26.478	26.776	C
Ibuprofen	2.181	−5.491	−5.720	11.979	12.477	C
Abacavir***	1.789		−6.562		11.740	C
Guaifenesin	1.211	−6.482	−5.820	7.848	7.047	N
Guaiacolsulfonate	0.991	−4.562	−5.482	4.519	5.430	C
Pseudoephedrine	0.669	−5.581	−5.348	3.736	3.579	N
Caffeine	0.463	−5.333	−5.128	2.471	2.377	N
Loxoprofen	0.168	−6.468	−6.184	1.086	1.038	N
Dextromethorphan	0.130	−6.537	−6.315	0.847	0.819	N
Noscapine	0.116	−7.464	−6.792	0.867	0.789	N
d-Methylephedrine	0.139	−6.029	−5.636	0.838	0.784	N
Ambroxol	0.109	−6.763	−6.247	0.734	0.678	N
Dihydrocodeine	0.060	−5.401	−5.709	0.325	0.343	C
Bromhexine	0.029	−6.602	−5.655	0.192	0.164	N
Carbinoxamine	0.018	−7.616	−6.073	0.140	0.112	N
Isopropamide	0.012	−7.729	−6.102	0.097	0.076	N
d-Chlorpheniramine	0.010	−7.265	−6.690	0.070	0.064	N
Mequitazine	0.012	−7.021	−5.021	0.087	0.062	N
Clemastine	0.003	−7.680	−6.265	0.022	0.018	N

*GBVI_dG: GBVI/WSA_dG. **Composite risk index: |GBVI/WSA_dG| × (maximum daily dose).
***Binding to the HLA-B*57:01 molecule.

scoring functions of GBVI/WSA_dG [11] which is considered to express protein-ligand binding free energy.

Redocking simulations were performed using the crystal structure of the complex between abacavir and the HLA-B*57:01 molecule (PDB ID: 3VRI) to assess the appropriateness of using ASEDock for the drug-HLA systems. The root-mean square deviation (rmsd) between nonhydrogen atoms of abacavir in the crystal and docked structures is 0.99 Å. Prediction within rmsd of 2.0 Å is held as the passing standard. The validation clearly indicated that ASEDock can simulate accurately enough the structure and position of the bound abacavir molecule in the crystal structure and the docking algorithm is suitable for the docking simulations of the drug-HLA systems. A superposition of the simulated and experimental structures is shown in Figure 2. The GBVI/WSA dG value is calculated to be −6.56 kcal/mol.

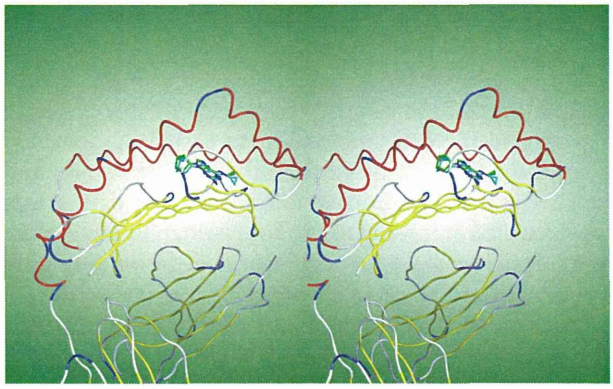
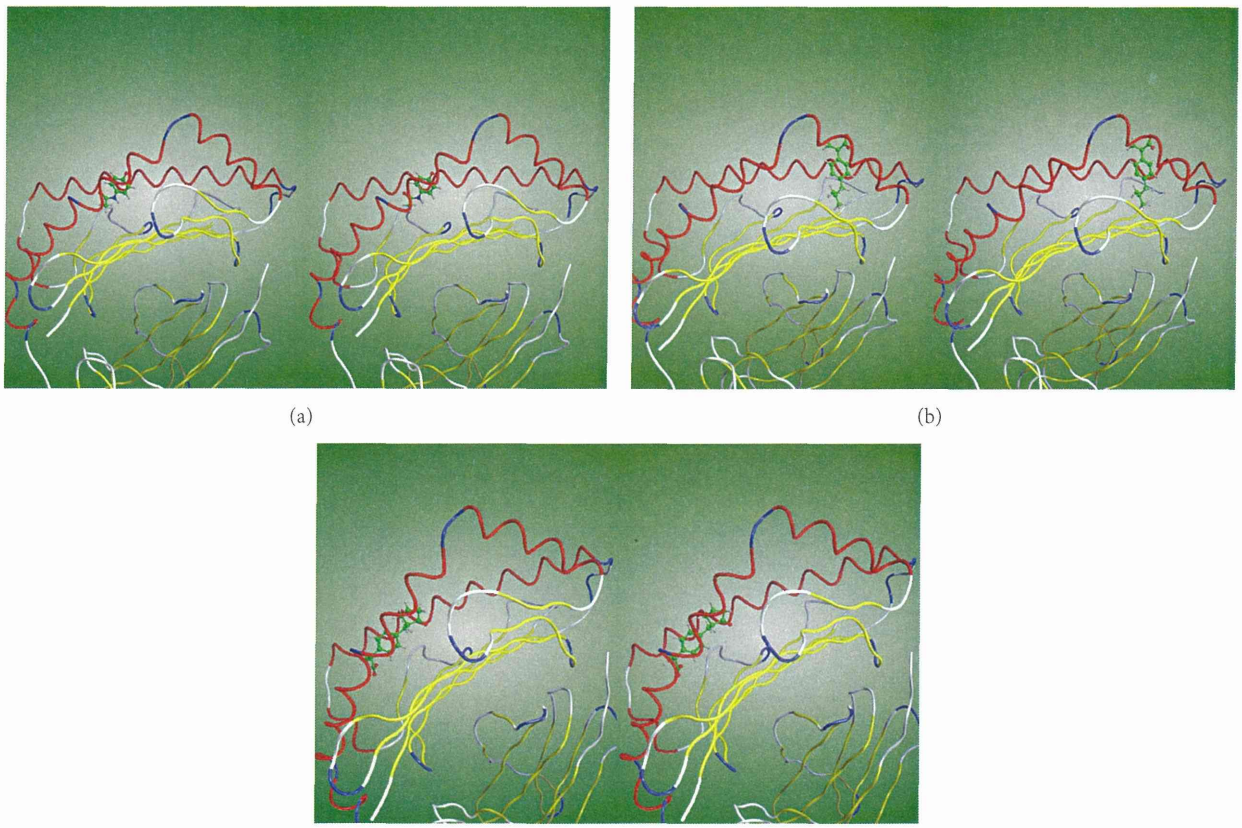


Figure 2: A superposition of simulated and experimental structures of abacavir. The drug is bound at the antigenic-peptide binding groove of the HLA-B*57:01 molecule. The HLA molecules are depicted schematically. The alpha helix and beta strand are shown by red and yellow tubes, respectively. The drug molecules are depicted by stick models. The carbon atoms of abacavir located by X-ray analysis and docking simulations are colored in cyan and green, respectively. This figure is a cross-eyed stereoscopic drawing.

3. Results and Discussion

The binding affinities to both sites are given in Table 2. As the racemates of methylephedrine and chlorpheniramine are used, the optical isomers with higher binding affinities are given in the table. Although the binding affinity to the HLA molecule would play an important role to trigger the following immunological response, the probability of occurrence of the adverse reactions might significantly depend on the dose of the drug. Therefore it may be reasonable to use a composite risk index (CRI) calculated by multiplying the absolute value of the binding affinity by the daily dose together to assess the



(a) (b) (c)

FIGURE 3: Cross-eyed stereoscopic drawings of the drugs bound at the antigenic-peptide binding grooves of ADR-associated HLA molecules. The drugs are represented by stick models with carbon atoms and bonds colored in green. The interaction modes of acetaminophen, ibuprofen, and loxoprofen at the peptide-binding groove of the HLA-A*02:06 molecule are shown in (a), (b), and (c), respectively.

potential risk of the adverse reactions. In Table 2, the CRI is given and the drug molecules are sorted by descending order of the CRI calculated from the binding affinity to the site C. The maximum dose of each drug per day in mmol was calculated according to the dose written in each package insert of cold medicines. For comparison, the binding affinity of abacavir to the HLA-B*57 molecule is given. Most of the drugs are preferentially bound at the site N. Drugs with higher CRIs are, however, inclined to bind preferentially at the site C as abacavir.

If we set a CRI threshold of, for example, 1.0 mmol·kcal/mol arbitrarily, nine drugs are judged to be high-risk drugs causing adverse drug reactions through binding to HLA-A*02:06. In Japan, warnings about potential side effects of SJS and TEN are explicitly stated in the ethical drug package inserts of acetaminophen, ibuprofen, and loxoprofen [12]. Cases of SJS/TEN caused by acetaminophen [13], ibuprofen [14], and loxoprofen [15] have been reported, albeit the associations with HLA-A*02:06 have not been noticed. Ethenzamide was reported as a causative drug of SJS in Japan [16]. Since it is highly possible that the SJS caused by these four drugs is associated with HLA-A*02:06, studies to verify the associations are urgently required to understand the molecular mechanisms of the adverse reactions and find countermeasures against them. As to tranexamic acid, guaifenesin, guaiacol, pseudoephedrine, and caffeine, they are not considered as significant causative drugs of SJS at least in Japan. However, 17, 4, and 2 SJS cases caused by guaifenesin, pseudoephedrine, and caffeine, respectively, have been reported from the FDA [17]. TEN caused by tranexamic acid was reported [18]. Although it is highly possible that these adverse drug reactions are associated with different alleles, rather high CRIs of these drugs imply the involvement of these drugs in SJS/TEN. As comprehensive analysis about causative factors of SJS has not been undertaken, unknown factors might be involved in the SJS cases. Under these circumstance, we believe that SJS-like symptoms caused by drugs with the high CRIs should be monitored carefully.

The drugs with the CRI being lesser than 1.0 have not been noticed as culprit drugs for SJS/TEN at least in Japan. The low incidence of SJS/TEN caused by these drugs in Japan supports that the SJS/TEN associated with HLA-A*02:06 should be mainly determined by CRI. Although certain numbers of SJS cases have been reported on drugs such as clemastine, mequitazine and bromhexine from the FDA, the causative alleles in these cases may be different from HLA-A*02:06.

Hypersensitivities against these drugs are described in the ethical drug package inserts in Japan.

Metabolites for each drug molecule have not been fully disclosed yet. It is possible that some of such metabolites rather than the parent drugs would play more important roles in certain cases of ADRs. Therefore further simulation studies including the metabolites will be essential and interesting in the more distant future. Nevertheless, these uncertainties do not preclude the usefulness of the simulation results obtained for the parent drugs in the context of a weight of evidence approach for the time being. The *in silico* risk assessment methodology as used in the present study will certainly play an important role in identifying testing needs, setting testing priorities, and above all attracting our attention to the potential toxicities of the relevant drugs.

The binding modes of acetaminophen, ibuprofen, and loxoprofen at the antigenic peptide-binding groove of the HLA-A*02:06 molecule are illustrated in Figures 3(a), 3(b), and 3(c), respectively. The binding mode of abacavir to the HLA-B*57:01 is shown in Figure 2. In the crystal structure [5], abacavir binds deep across the bottom of the antigen-binding groove of the HLA molecule. Therefore new endogenous peptides can bind on top of the bound abacavir leading to the adverse drug reaction of abacavir. On the other hand, acetaminophen and loxoprofen are bound near the surface of the HLA molecule. Although one end of the ibuprofen molecule is bound to the bottom of the groove, the carboxyl group is exposed on the surface of the HLA molecule. Since the binding modes of these drugs appear different from that of abacavir, it is not clear whether new endogenous peptides can bind on top of the bound drugs. Hence further studies to confirm the molecular mechanisms leading to the ADRs are required.

4. Conclusions

Prompt recovery from SJS/TEN upon withdrawal of the relevant drugs indicates the importance of early diagnosis. However, identification of the culprit drugs is usually intractable especially in the cases where patients take drugs with multiple ingredients such as cold medicines. In these situations, a comprehensive risk assessment taking both of circumstantial evidence and theoretically deduced risk index into account would be helpful. In the present study we have shown that docking simulations between drug molecules and the HLA-A*02:06 molecule can explain the episodes of culprit drugs of SJS/TEN reported so far in Japan. In addition, the present study has pointed out the potential risk of several drugs whose involvement in SJS/TEN has not been explicitly noticed until now. This study urges further investigations of verifying the ADRs of these drugs in detail.

Conflict of Interests

The authors declare that they have no conflict of interests.

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