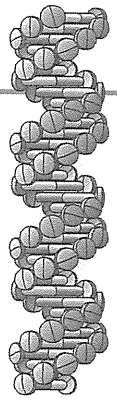

Specific HLA types are associated with antiepileptic drug-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese subjects

Aim: This preliminary study investigated genomic biomarkers for Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), related to three antiepileptic drugs, zonisamide, phenobarbital and phenytoin. **Patients & methods:** *HLA* class I and *HLA-DRB1* loci were genotyped for Japanese patients with zonisamide-, phenobarbital- or phenytoin-induced SJS/TEN (n = 12, 8 and 9, respectively) and for healthy Japanese volunteers (n = 2878). **Results:** Carrier frequencies of *HLA-A*02:07* in patients with zonisamide-induced SJS/TEN and in the general Japanese population were 41.7 and 6.81%, respectively. Carrier frequencies of *HLA-B*51:01* in patients with phenobarbital- and phenytoin-induced SJS/TEN and in controls were 75.0, 55.6 and 15.2%, respectively. *HLA-A*02:07* and *HLA-B*51:01*, in a dominant model, were significantly associated with zonisamide- and phenobarbital-induced SJS/TEN, respectively (Pc = 0.0176 and 0.0042, respectively). **Conclusion:** Our data suggest that *HLA-A*02:07* and *HLA-B*51:01* are potential biomarkers for zonisamide- and phenobarbital-induced SJS/TEN, respectively, in Japanese individuals.

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KEYWORDS: aromatic antiepileptic drug ***HLA-A*02:07*** ***HLA-B*51:01*** **phenobarbital phenytoin Stevens–Johnson syndrome toxic epidermal necrolysis zonisamide**

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Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe, life-threatening adverse drug reactions. SJS and TEN are characteristic mucosal disorders and are thought to represent different severities of the same disease [1,2]. The most widely accepted classification for SJS and TEN is based on the size of the skin detachment area, as follows: for SJS, a skin detachment area less than 10% of the body surface area; for a SJS–TEN overlap, from 10 to less than 30% of the body surface area; and for TEN, no less than 30% of the body surface area [1]. In Japan, TEN is defined as a skin detachment area more than 10% of the body surface area [3]. Although the incidence of SJS and TEN is very low, the mortality rates for these diseases are estimated to be 1–5 and 20–30%, respectively [4,5].

Recently, the development of carbamazepine-induced SJS/TEN was shown to involve *HLA-B*15:02* in Southeast Asian patients [6–12] and individuals of Asian descent living in Europe [13], while other phenotypes of carbamazepine-induced cutaneous adverse reactions (CARs), such as hypersensitivity syndrome and maculopapular exanthema, are not associated with *HLA-B*15:02* [11,14]. *HLA-B*15:02* was also reported to be associated with phenytoin- and oxcarbazepine-induced SJS/TEN in Han Chinese and Thai patients [11,15]. For Japanese and Korean patients, although carbamazepine-induced SJS/TEN shows no significant association with *HLA-B*15:02*, it is significantly associated with *HLA-B*15:11*, which belongs to the same serotype, HLA-B75, as *HLA-B*15:02* [16,17]. In addition, another genetic biomarker, *HLA-A*31:01*, has recently been reported to be strongly associated with carbamazepine-induced SJS/TEN and other types of CARs in both Japanese and Caucasian individuals [18–20]. However, this marker has previously been reported to be associated with hypersensitivity syndrome and maculopapular exanthema caused by carbamazepine in Han Chinese individuals and is not associated with SJS/TEN [14]. Associations between *HLA-B*58:01* and allopurinol-induced severe CARs, including SJS/TEN and hypersensitivity, have been recognized in various ethnic groups, such as Asians [21–24] and Europeans [13].

While the number of genomic biomarkers associated with the onset of CARs has been increasing, genomic biomarkers have only been identified for the limited number of causative drugs mentioned above and for anti-HIV drugs, such as abacavir [25] and nevirapine [26]. Therefore, in the current preliminary study in Japanese patients, we examined biomarkers for SJS/TEN caused by phenytoin, zonisamide or phenobarbital, which are often used for epilepsy treatment in Japan and are known to contribute to the development of SJS/TEN [27].

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Patients & methods

Patients

Unrelated Japanese patients who experienced SJS or TEN caused by phenytoin, zonisamide or phenobarbital were recruited from two separate groups: the Japan Severe Adverse Reactions (JSAR) research group and RIKEN. In the JSAR research group, patients were recruited between June 2006 and July 2012 from participating institutions or through a nationwide blood sampling network in Japan operated by the National Institute of Health Sciences in cooperation with: the Ministry of Health, Labour and Welfare; Pharmaceutical and Medical Devices Agency; and Federation of Pharmaceutical Manufacturers' Association of Japan [16]. In RIKEN, patients were registered in BioBank Japan [28]. Both groups applied the same criteria proposed by Bastuji-Garin *et al.* for the diagnosis of SJS/TEN [1]; however, the SJS–TEN overlap was categorized as TEN according to the severity criteria used in Japan in this study [3]. Aromatic antiepileptic drugs that were administered to patients within 2 months before the onset of SJS/TEN were assumed to be causative drugs, although some coadministered drugs could not be excluded as the culprit drugs in some patients (TABLE 1). Potential drug–drug interactions caused by the coadministered drugs were ruled out since we did not find any strong CYP2C9 (for phenytoin and phenobarbital) or CYP3A4 (for zonisamide) inhibitors among them. The other patients' background information, such as demographic characteristics, phenotypes of adverse reactions, antiepileptic drugs administered within 2 months before the onset of SJS/TEN and primary diseases are also summarized in TABLE 1. Nine patients had phenytoin-related SJS/TEN. Twelve patients had zonisamide-related SJS/TEN; two of these patients were included in our previous study [16], and another patient (P7) also received phenytoin and was included in the association studies for phenytoin-induced SJS/TEN. Eight patients had phenobarbital-related SJS/TEN, one of whom (P5) had also received phenytoin and was included in the association studies for phenytoin-induced SJS/TEN. For the control group, healthy Japanese volunteers living in Japan were recruited by the Japan Pharmacogenomics Data Science Consortium (JPDSC). Control data obtained from volunteers from Okinawa prefecture (the southernmost islands of Japan) were excluded as principal component analysis by EIGENSOFT ver 4.0 (Helix Systems, MD, USA) using 332,535 SNPs obtained from a genome-wide association study using Illumina Human Omni2.5 BeadChip (Illumina, Inc., CA, USA) showed that subjects from Okinawa prefecture were separated from subjects from other regions in Japan, and also because none of the patients in this study were from hospitals in Okinawa prefecture. We used data obtained from 2878 healthy Japanese volunteers as control data. The ethics committees of the participating institutions approved this study. Written informed consent was obtained from each subject.

HLA typing

For the JSAR research group, high-resolution *HLA* typing was performed with a sequence-based method using SeCore A, SeCore B, SeCore C and SeCore DRB1 Locus Sequencing Kits (Invitrogen Corp., WI, USA) and ABI 3730 and 3130 DNA sequencers (Applied Biosystems, CA, USA). HLA types were estimated using the Assign SBT or Assign ATF software (version 3.2.7b and version 1.0.2.41, respectively; Conexio Genomics, Western Australia, Australia). For RIKEN and JPDSC, high-resolution *HLA* typing was performed using HLA-typing kits (WAKFlow, Hiroshima, Japan; or LABType SSO, One Lambda, CA, USA), which are based on PCR sequence-specific oligonucleotide probes coupled with multiple analyte profiling (xMAP) technology (Luminex System; Luminex Corporation, TX, USA). Haplotype analysis for control subjects was conducted using LDSUPPORT [29] modified for multiallelic data, and frequencies for individual haplotypes were counted.

Statistical analysis

A total of 17 and 14 *HLA* genotypes were detected in multiple patients in the phenytoin and phenobarbital studies, respectively. For these *HLA* genotypes, frequencies in the SJS/TEN groups were compared with those of controls using a dominant genotyping model, and p-values were corrected for multiplicity of testing by the *HLA* genotype numbers compared in individual studies (Bonferroni's correction). In the zonisamide study, frequencies of 21 *HLA* genotypes detected in multiple patients and a haplotype were compared, and p-values were corrected according to the total number (22) of comparisons. For a control group, we used healthy Japanese volunteers rather than patients tolerant to antiepileptic drugs as the incidence of SJS/TEN in Japanese individuals is very low (~3–3.5

Table 1. Backgrounds of Japanese patients with antiepileptic drug-induced Stevens–Johnson syndrome/toxic epidermal necrolysis and their four-digit HLA types.

Patient ID	ADR phenotype	Aromatic antiepileptics administered	Sex/age (years)	Primary disease	Period of latency (days)	Potential other causative drugs†	*HLA-A*	*HLA-B*	*HLA-C*	*HLA-DRB1*
P1	SJS	PHT	M/8	Rasmussen syndrome	11	Gabapentin	*24:02/26:03*	*40:01/51:01*	*03:04/14:02*	*09:01/11:01*
P2	TEN	PHT	M/4	Intractable epilepsy	16	None	*26:01/31:01*	*44:03/51:01*	*14:02/14:03*	*13:02/14:03*
P3	SJS	PHT	M/5	Epilepsy	15	None	*11:01/24:02*	*55:04/67:01*	*03:03/07:02*	*09:01/15:01*
P4	TEN	PHT	F/73	Epilepsy	5	Tazobactam Piperacillin sodium	*26:01/26:02*	*40:01/51:01*	*01:02/03:04*	*04:05/14:05*
P5	TEN	PHT/PB	F/41	Unavailable	~12	None	*11:01/24:20*	*51:01/55:02*	*01:02/15:02*	*04:05/15:01*
P6	SJS	PHT	M/67	Unavailable	30	Minocycline hydrochloride Tiapride hydrochloride	*02:07/24:02*	*40:01/46:01*	*01:02/07:02*	*08:03/08:03*
P7	SJS	PHT/ZNS	F/31	Symptomatic epilepsy	16	Sodium valproate	*02:01/02:01*	*15:18/38:02*	*07:02/08:01*	*11:01/13:02*
P8	TEN	PHT	F/64	Convulsions after γ-knife treatment	1	None	*02:01/24:02*	*15:01/51:01*	*04:01/14:02*	*08:03/09:01*
P9	TEN	PHT	F/56	Depression	16	Sodium valproate	*11:01/11:01*	*15:01/54:01*	*01:02/04:01*	*04:05/04:06*
P101	SJS	ZNS	F/71	Symptomatic epilepsy after brain infarction	21	Omeprazole Candesartan cilexetil	*31:01/31:01*	*40:02/51:01*	*03:04/14:02*	*08:02/09:01*
P102	SJS	ZNS	F/57	Symptomatic epilepsy after surgery	8	None	*02:07/26:01*	*40:03/46:01*	*01:02/03:04*	*08:03/15:02*
P103	SJS	ZNS	M/25	Epilepsy	20	None	*02:07/31:01*	*13:01/46:01*	*01:02/03:03*	*08:03/12:02*
P104	TEN	ZNS/CBZ	M/17	Epilepsy	33	Amoxicillin hydrate	*02:07/31:01*	*46:01/56:01*	*01:02/04:01*	*08:03/09:01*

†*Drugs other than aromatic antiepileptics that were coadministered no more than 2 months prior to the onset of SJS/TEN.*
ADR: Adverse drug reaction; CBZ: Carbamazepine; F: Female; M: Male; P: Patient; PB: Phenobarbital; PHT: Phenytoin; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis; ZNS: Zonisamide.

Table 1. Backgrounds of Japanese patients with antiepileptic drug-induced Stevens–Johnson syndrome/toxic epidermal necrolysis and their four-digit HLA types (cont.).

Patient ID	ADR phenotype	Aromatic antiepileptics administered	Sex/age (years)	Primary disease	Period of latency (days)	Potential other causative drugs[†]	*HLA-A*	*HLA-B*	*HLA-C*	*HLA-DRB1*
P105	TEN	ZNS	M/52	Symptomatic epilepsy after brain bleeding	32	Valsartan Lansoprazole	*02:07/24:02*	*35:01/46:01*	*01:02/03:03*	*08:03/14:05*
P106	TEN	ZNS	M/78	Symptomatic epilepsy after brain bleeding	22	None	*02:01/24:02*	*39:01/67:01*	*07:02/07:02*	*09:01/12:01*
P107	SJS	ZNS	M/6	Epilepsy	24	None	*24:02/26:03*	*15:11/40:06*	*03:03/08:01*	*09:01/14:02*
P7	SJS	PHT/ZNS	F/31	Symptomatic epilepsy	40	Sodium valproate	*02:01/02:01*	*15:18/38:02*	*07:02/08:01*	*11:01/13:02*
P108	SJS	ZNS	M/63	Symptomatic epilepsy because of glioblastoma	18	None	*02:07/24:02*	*46:01/52:01*	*01:02/12:02***	*08:03/15:02*
P109	SJS	ZNS	F/30	Symptomatic epilepsy after surgery	18	Sodium valproate	*24:02/24:02*	*51:01/54:01*	*01:02/14:02*	*04:05/12:01*
P110	SJS	ZNS	M/56	Symptomatic epilepsy because of brain cancer	30	None	*02:06/24:02*	*07:02/52:01*	*07:02/12:02*	*01:01/15:02*
P111	SJS	ZNS	M/59	Symptomatic epilepsy after head injury	28	None	*24:02/26:01*	*40:02/52:01*	*03:04/12:02*	*08:02/15:02*
P201	SJS	PB	M/6	Epilepsy	14	None	*24:02/24:02*	*15:01/51:01*	*03:04/04:01*	*04:06/09:01*
P202	SJS	PB	M/69	Epilepsy	11	None	*24:20/26:03*	*15:01/51:01*	*03:03/14:02*	*04:03/04:10*
P203	TEN	PB	F/42	Symptomatic epilepsy because of glioblastoma	29	Temozolomide	*24:02/24:02*	*51:01/54:01*	*01:02/14:02*	*09:01/13:01*
P204	TEN	PB	F/26	Epilepsy	10	None	*26:01/26:01*	*40:02/40:06*	*03:04/08:01*	*04:10/09:01*
P205	SJS	PB	F/67	Symptomatic epilepsy after head injury	20	Candesartan cilexetil Famotidine	*02:01/26:01*	*15:01/51:01*	*08:01/15:02*	*12:02/14:01*

[†]Drugs other than aromatic antiepileptics that were coadministered no more than 2 months prior to the onset of SJS/TEN.
ADR: Adverse drug reaction; CBZ: Carbamazepine; F: Female; M: Male; P: Patient; PB: Phenobarbital; PHT: Phenytoin; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis; ZNS: Zonisamide.

Table 1. Backgrounds of Japanese patients with antiepileptic drug-induced Stevens–Johnson syndrome/toxic epidermal necrolysis and their four-digit HLA types (cont.).

Patient ID	ADR phenotype	Aromatic antiepileptics administered	Sex/age (years)	Primary disease	Period of latency (days)	Potential other causative drugs†	*HLA-A*	*HLA-B*	*HLA-C*	*HLA-DRB1*
P5	TEN	PHT/PB	F/41	Unavailable	9	None	*11:01/24:20*	*51:01/55:02*	*01:02/15:02*	*04:05/15:01*
P206	TEN	PB	F/28	Unavailable	Unavailable	None	*02:06/24:02*	*51:01/51:01*	*14:02/14:02*	*04:05/04:05*
P207	SJS	PB	M/3	Localization-related epilepsy	13	None	02:01/33:03	39:04/44:03	*07:02/14:03*	*04:03/13:02*

†Drugs other than aromatic antiepileptics that were coadministered no more than 2 months prior to the onset of SJS/TEN.
ADR: Adverse drug reaction; CBZ: Carbamazepine; F: Female; M: Male; P: Patient; PB: Phenobarbital; PHT: Phenytoin; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis; ZNS: Zonisamide.

patients/million/year) [30], and the control subjects were therefore highly unlikely to develop SJS/TEN throughout their entire lives. The use of healthy controls provides more conservative results than the use of tolerant patient controls. Fisher's exact test was conducted using JMP version 7.0.1 (SAS Institute Japan Ltd), and odds ratios and 95% CIs were calculated using the same software.

Results

SJS/TEN patients with *HLA* class I and *HLA-DRB1* recruited in this study are shown in Table 1. Nine patients developed SJS/TEN within 60 days after the start of phenytoin treatment, and the average latency after the first exposure to phenytoin was 13.6 ± 8.1 days. Seventeen *HLA* types were detected in multiple patients and carrier frequencies were compared with those of healthy Japanese controls for these alleles. Five of nine patients with phenytoin-induced SJS/TEN carried *HLA-B*51:01*, and the carrier and allelic frequencies in these cases (55.6 and 27.8%, respectively) were considerably higher than those in the controls (15.2 and 7.87%, respectively; Table 2). However, the association between *HLA-B*51:01* and phenytoin-induced SJS/TEN was not significant after adjustment for multiplicity of testing (corrected P by Bonferroni's method [Pc] = 0.1037 in a dominant mode). No other alleles showed a remarkable association with phenytoin-induced SJS/TEN.

Twelve patients had SJS/TEN for which zonisamide was a causative drug. The average latency after the first exposure to zonisamide was 23.8 ± 8.5 days. *HLA-A*02:07*, *HLA-B*46:01* and *HLA-DRB1*08:03* were associated with zonisamide-induced SJS/TEN in a dominant mode (p = 0.0008, odds ratio: 9.77 [95% CI: 3.07–31.1]; p = 0.0037; odds ratio: 6.73 [95% CI: 2.12–21.36]; and p = 0.0306, odds ratio: 3.78 [95% CI: 1.20–11.97], respectively), although only *HLA-A*02:07* was significantly associated with the disease after Bonferroni's correction (Pc = 0.0176). These alleles, that is, *HLA-A*02:07*, *HLA-B*46:01* and *HLA-DRB1*08:03*, appeared to be linked to each other, as shown in Table 1. Indeed, the haplotype *HLA-A*02:07_HLA-B*46:01_HLA-C*01:02_HLA-DRB1*08:03* has been previously reported by Saito *et al.* [31]. Among the alleles constituting this haplotype, only *HLA-C*01:02* did not show a statistically significant association with zonisamide-induced SJS/TEN, even before Bonferroni's correction, because of its high allelic frequency in the controls (17.1%; data not shown).

The haplotype frequency in SJS/TEN patients (5/24; 20.8%) was significantly higher than that in controls (2.9%; p = 0.0001; odds ratio: 12.36 [95% CI: 4.54–33.65]; Pc = 0.0021). No other *HLA* genotypes or haplotypes showed a remarkable association with zonisamide-induced SJS/TEN.

Phenobarbital was a causative drug for eight patients with SJS/TEN. The average latency after the initiation of treatment with phenobarbital was 15.1 ± 7.1 days, which was slightly longer and approximately 1 week shorter than those for phenytoin- and zonisamide-induced SJS/TEN, respectively. The results of association studies between phenobarbital-induced SJS/TEN and the HLA types are summarized in Table 2. Six of eight SJS/TEN patients carried *HLA-B*51:01* and an association between phenobarbital-induced SJS/TEN and *HLA-B*51:01* was observed (p = 0.0003; odds ratio: 16.71 [95% CI: 3.66–83.06]). This association was still significant after Bonferroni's correction (Pc = 0.0042). Carrier frequencies of other *HLA* types, that is, *HLA-A*24:20*, and *HLA-DRB1*04:10*, were also higher in the SJS/TEN patient group than in the healthy volunteers, although the associations were not significant after the correction. Despite the very low allelic frequency of *HLA-A*24:20* in the Japanese population (0.834%), we found two patients carrying this *HLA* type, and both patients also carried *HLA-B*51:01*.

Discussion

Researchers have been discovering an increasing number of genomic biomarkers associated with CARs. For carbamazepine-induced CARs, *HLA-B75* (which includes *HLA-B*15:11*, *HLA-B*15:08* and *HLA-B*15:21* as well as *HLA-B*15:02*) and *HLA-A*31:01* have been reported as biomarkers [6–14,16–20]. In this study, we investigated genetic biomarkers for phenytoin-, zonisamide- and phenobarbital-induced SJS/TEN and, despite small sample sizes, found two completely different risk factors to those for carbamazepine-induced CARs; *HLA-A*02:07* and *HLA-B*51:01* were found to be significantly associated with SJS/TEN induced by other aromatic antiepileptic drugs, that is, zonisamide and phenobarbital, in Japanese patients.

In this study, the haplotype *HLA-A*02:07_HLA-B*46:01_HLA-C*01:02_HLA-DRB1*08:03* and the single allele *HLA-A*02:07* were found to be significantly associated with zonisamide-induced SJS/TEN. A borderline p-value was also obtained for the association with *HLA-B*46:01*, based on a dominant mode analysis, although the association was not

Table 2. Major associations between HLA types and antiepileptic drug-induced Stevens–Johnson syndrome/toxic epidermal necrolysis observed in this study.

Allele	Healthy Japanese volunteers		SJS/TEN		Dominant genotypic model	
	Allele frequency (%)	*Carriers, n (%)*	*Allele frequency (%)*	*Carriers, n (%)*	*p-value Pc-value*	*Odds ratio (95% CI)*
Phenytoin-induced SJS/TEN						
*HLA-B*51:01*	438/5756 (7.87)	438/2878 (15.22)	5/18 (27.78)	5/9 (55.56)	p = 0.0061 Pc = 0.1037	6.96 (1.86–26.03)
Zonisamide-induced SJS/TEN						
*HLA-A*02:07*	201/5756 (3.49)	196/2878 (6.81)	5/24 (20.83)	5/12 (41.67)	p = 0.0008 Pc = 0.0176	9.77 (3.07–31.1)
*HLA-B*46:01*	286/5756 (4.97)	276/2878 (9.59)	5/24 (20.83)	5/12 (41.67)	p = 0.0037 Pc = 0.0814	6.73 (2.12–21.36)
*HLA-DRB1*08:03*	475/5756 (8.25)	457/2878 (15.88)	5/24 (20.83)	5/12 (41.67)	p = 0.0306 Pc = 0.6732	3.78 (1.20–11.97)
Phenobarbital-induced SJS/TEN						
*HLA-A*24:20*	48/5756 (0.83)	47/2878 (1.46)	2/16 (12.50)	2/8 (25.00)	p = 0.0074 Pc = 0.1036	20.08 (3.95–102.07)
*HLA-B*51:01*	453/5756 (7.87)	438/2878 (15.22)	7/16 (43.75)	6/8 (75.00)	p = 0.0003 Pc = 0.0042	16.71 (3.66–83.06)
*HLA-DRB1*04:10*	115/5756 (2.00)	114/2878 (3.96)	2/16 (12.50)	2/8 (25.00)	p = 0.0383 Pc = 0.5362	8.08 (1.61–40.48)

Pc: Corrected p-value by Bonferroni's method; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

significant after Bonferroni's correction. Therefore, *HLA-A*02:07* or *HLA-B*46:01* rather than *HLA-C*01:02* and *HLA-DRB1*08:03* may be responsible for the development of zonisamide-induced SJS/TEN in Japanese patients. The allelic frequencies of *HLA-A*02:07* in Japanese, Korean and Chinese individuals are 0.07–2.2, 3.0 and 0–22.7%, respectively, and the frequencies are very low in Caucasian and African individuals (0.0–1.0%) [32]. The allelic frequencies of *HLA-B*46:01* in Japanese, Korean, Chinese, Caucasian and African individuals are 1.0–6.1, 4.4, 0.0–25.4, 0.0–0.7 and 0.0–3.1%, respectively [32]. Strong associations between *HLA-B*59:01* and SJS/TEN caused by methazolamide and acetazolamide, which are sulfonamide derivatives used as carbonic anhydrase inhibitors for lowering intraocular pressure in glaucoma, have been reported in Japanese [33] and Korean patients [34,35]. Although zonisamide has a sulfonamide structure, as shown in SUPPLEMENTARY FIGURE 1 (see www.futuremedicine.com/doi/suppl/10.2217/PGS.13.180), no patients with SJS/TEN caused by zonisamide carried *HLA-B*59:01* in this study.

We found an association between phenobarbital-induced SJS/TEN and *HLA-B*51:01*, even after the correction for multiple testing. This allele was also marginally associated with phenytoin-induced SJS/TEN in analyses based on a dominant model ($p = 0.0061$; $Pc = 0.1037$; TABLE 2). The allelic frequency of *HLA-B*51:01* has also been reported to be higher in Han Chinese patients in Taiwan with phenytoin-induced SJS/TEN than in a tolerant control group, although the association was not statistically significant [15]. As shown in SUPPLEMENTARY FIGURE 1, phenobarbital and phenytoin have a very similar chemical structure that are very different from the structures of carbamazepine and zonisamide. Phenobarbital and phenytoin are heterocyclic compounds with two nitrogen atoms embedded in their rings that are substituted with at least two carbonyl groups and with at least one phenyl group. Therefore, it is not surprising that the same HLA molecule type is potentially involved in the onset of SJS/TEN caused by phenobarbital and phenytoin. The same mechanism, such as direct interactions with *HLA-B*51:01* molecules, may be involved in the pathogenesis of SJS/TEN for these two drugs, as previously shown for carbamazepine-induced SJS/TEN [36]. Thus, *HLA-B*51:01* may be a risk factor for SJS/TEN caused by phenytoin and phenobarbital in Asian individuals.

Three HLA types, that is, *HLA-A*02:07*, *HLA-B*46:01* and *HLA-B*51:01*, which were associated with zonisamide- or phenobarbital-induced SJS/TEN, were also detected in patients with SJS/TEN caused by the other drugs collected by the JSAR research group, but their allelic frequencies were similar to those in healthy volunteers (data not shown). Since three of the five carriers of *HLA-A*02:07* and three of the nine carriers of *HLA-B*51:01* were patients with secondary epilepsy caused by various conditions, such as brain bleeding, brain surgery, brain injury or brain tumors (TABLE 1), these alleles have been suggested to be risk factors for SJS/TEN rather than biomarkers for epilepsy.

As shown in TABLE 2, the allele frequencies of *HLA-B*51:01*, *HLA-A*02:07* and *HLA-B*46:01* in our Japanese population were 7.87, 3.49 and 4.97%, respectively, and they were therefore relatively common alleles in Japanese individuals. As the incidence of SJS/TEN caused by these three drugs is very low, as assumed from the patient numbers reported to the regulatory agency according to the pharmaceutical affairs law, most patients carrying these alleles will not develop SJS/TEN when they receive these drugs. This discrepancy between the incidence of adverse reactions and biomarker allele frequencies is also seen in the case of carbamazepine-induced SJS/TEN in Taiwan, where the allele frequency of *HLA-B*15:02* is relatively high (8.6%) [6] and the incidence of carbamazepine-induced SJS/TEN among patients to whom the drug was newly given was very low (0.22–0.24%) [37]. For the case of carbamazepine-induced SJS/TEN, biased usage of a specific repertoire of the third complementarity-determining region of the T-cell receptor, VB-11-ISGSY, is associated with the development of SJS/TEN in addition to carrying *HLA-B*15:02* [38]. In the development of SJS/TEN induced by zonisamide, phenobarbital or phenytoin, other risk factors, which could be specific to culprit drugs and phenotypes of adverse reactions, may also be required. To elucidate these factors, *in vitro* or clinical studies using blister fluid or T-cells obtained from patients are necessary.

Sensitivities of *HLA-A*02:07* (41.7%) in zonisamide-induced SJS/TEN and *HLA-B*51:01* (55.6%) in phenytoin-induced SJS/TEN were not as high as those observed in carbamazepine-induced SJS/TEN and allopurinol-induced CARs observed in Taiwanese

patients (98–100%) [6,21]; the reasons for this are unclear. However, the sensitivities of the biomarkers observed in this study are nearly comparable with those of *HLA-B*58:01* in patients with allopurinol-induced SJS/TEN in Japan (55.6%) [24] and in Europe (55.6%) [13], and with those of *HLA-A*31:01* in Japanese and European patients with carbamazepine-induced CARs (58.4 and 27.3%, respectively) [19,20]. The clinical usefulness of biomarkers that show such moderate associations with adverse reactions remains inconclusive.

The results obtained in this study may have limited impact due to the very small sample size. Performance characteristics of *HLA-A*02:07* and *HLA-B*51:01*, such as positive predictive value, negative predictive value or number of patients needed to test, could not be calculated or estimated since this was a retrospective case–control study and the prevalence of SJS/TEN caused by these aromatic antiepileptic drugs in Japan is not known. Furthermore, we could not rule out the effects of population stratification on our results. Independent replication studies are necessary to confirm the roles of these *HLA* types detected in our study in the development of SJS/TEN induced by zonisamide, phenobarbital or phenytoin.

Conclusion

Our exploratory study suggested associations of the haplotype *HLA-A*02:07_HLA-B*46:01_HLA-C*01:02_HLA-DRB1*08:03* and the allele *HLA-A*02:07* with zonisamide-induced SJS/TEN, and an association of the allele *HLA-B*51:01* with phenobarbital-induced SJS/TEN in Japanese patients. The involvement of these alleles in the development of SJS/TEN should be confirmed by independent replication studies with larger sample sizes as well as *in vitro* studies, such as binding studies with HLA molecules expressed in cells.

Future perspective

Genomic biomarkers showing an association with the onset of CARs have been identified, and personalized medicine has started identifying patients at high risk of severe CARs based on pharmacogenomics by using biomarkers, such as *HLA-B*15:02* and *HLA-B*57:01*. However, among more than 100 causative drugs, including aromatic antiepileptic drugs, biomarkers have only been identified for a select few, such as carbamazepine, allopurinol and abacavir. Therefore, more intensive, nationwide or even international case–control studies are necessary to identify corresponding biomarkers that can predict patients at high risk on the basis of ethnicity and the causative drug. The accumulation of such data may help to uncover the pathogenic mechanisms of SJS/TEN, which will be useful for determining the safety of new molecules at early stages of the drug-development process and, thus, contribute to the development of new treatments for, or prevention of, severe CARs.

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Executive summary

Background

- Recently, the serotype HLA-B75 has been reported to be a risk factor for carbamazepine-induced SJS/TEN in Asians, and *HLA-A*31:01* has been reported to be a risk factor for carbamazepine-induced severe cutaneous adverse reactions, including SJS/TEN, in both Asians and Europeans.
- *HLA-B*15:02* is associated with phenytoin-induced SJS/TEN in Han Chinese patients in Taiwan.
- We conducted a case–control study investigating genetic biomarkers related to antiepileptic drug-induced SJS/TEN in Japanese patients.

Methods

- *HLA* class I and *HLA-DRB1* loci were genotyped in Japanese patients with zonisamide-, phenobarbital- or phenytoin-induced SJS/TEN and in healthy Japanese volunteers.

Results

- In dominant genetic models, *HLA-A*02:07* and *HLA-B*51:01* were associated with zonisamide- and phenobarbital-induced SJS/TEN, respectively, in Japanese patients.

Conclusion

- *HLA-A*02:07* and *HLA-B*51:01* are potential risk factors for zonisamide- and phenobarbital-induced SJS/TEN in Japanese patients.
- Independent replication studies are necessary to confirm the roles of these HLA types in the development of SJS/TEN induced by these aromatic antiepileptic drugs.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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ORIGINAL ARTICLE

Rebamipide Suppresses PolyI:C-Stimulated Cytokine Production in Human Conjunctival Epithelial Cells

Mayumi Ueta, Chie Sotozono, Norihiko Yokoi, and Shigeru Kinoshita

Abstract

Purpose: We previously documented that ocular surface epithelial cells could regulate ocular surface inflammation and suggested that, while Toll-like receptor 3 upregulates, EP3, one of the prostaglandin E_2 receptors, downregulates ocular surface inflammation. Others reported that rebamipide, a gastroprotective drug, could not only increase the gastric mucus production, but also suppressed gastric mucosal inflammation and that it was dominantly distributed in mucosal tissues. The eyedrop form of rebamipide, approved in Japan for use in the treatment of dry eye diseases, upregulates mucin secretion and production, thereby suppressing superficial punctate keratopathy on the ocular surface of patients with this disease. In the current study, we investigated whether rebamipide has anti- inflammatory effects on the ocular surface.

Methods: To examine the effects of rebamipide on polyI:C-induced cytokine expression by primary human conjunctival epithelial cells, we used enzyme-linked immunosorbent assay and quantitative reverse transcription-polymerase chain reaction assay. We studied the effects of rebamipide on ocular surface inflammation in our murine experimental allergic conjunctivitis (EAC) model.

Results: Rebamipide could suppress polyI:C-induced cytokine production and the expression of mRNAs for CXCL10, CXCL11, RANTES, MCP-1, and IL-6 in human conjunctival epithelial cells. In our EAC model, the topical administration of rebamipide suppressed conjunctival allergic eosinophil infiltration.

Conclusions: The topical application of rebamipide on the ocular surface might suppress ocular surface inflammation by suppressing the production of cytokines by ocular surface epithelial cells.

Introduction

The stimulation Toll-like receptor (TLR) 3 by polyI:C induces the secretion of inflammatory cytokines such as IL-6, IL-8, and MCP-1, type I IFN such as IFN-β, IFN-inducible proteins such as CXCL10 and CXCL11, and allergy-related proteins such as RANTES in human ocular surface epithelial cells.[1,2] This suggests that TLR3 of ocular surface epithelial cells upregulates ocular surface inflammation. On the other hand, prostaglandin E_2 (PGE_2) and its receptors, EP2 and EP3, downregulate the production of polyI:C-induced cytokines in human ocular surface epithelial cells.[3,4] Elsewhere we reported that TLR3 positively regulates the late-phase reaction in experimental allergic conjunctivitis (EAC),[5] that PGE_2 acts as a ligand for EP3 in conjunctival epithelial cells, and downregulates the progression of murine EAC.[6] We subsequently documented that EP3 negatively regulates the infiltration of eosinophils in EAC induced by TLR3, resulting in reduced eosinophilic conjunctival inflammation in TLR3/EP3 DKO mice, although EP3 KO mice manifested pronounced eosinophilic conjunctival inflammation.[7] These findings indicate that ocular surface epithelial cells could regulate ocular surface inflammation.

Rebamipide, a gastroprotective drug, has been prescribed for the treatment of gastric ulcers and gastritis. The drug has been reported to increase gastric mucus production[8,9] and to suppress gastric mucosal inflammation.[10,11] According to Naito et al. (1996), rebamipide was dominantly distributed in mucosal tissues; its mean mucosal concentration after ingestion was more than 100 times its mean serum concentration.[12]

In Japan, rebamipide in an eyedrop form has been approved for use in the treatment of dry eye disease. As the drug upregulates the production and secretion of mucin, it helps to suppress superficial punctate keratopathy on the ocular surface in patients with this disease.

In the current study, we investigated whether rebamipide could suppress cytokine production in human conjunctival epithelial cells, and whether it could suppress ocular surface inflammation in our murine EAC model.

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Materials and Methods

Human conjunctival epithelial cells

Our study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients after they were given a detailed explanation of the purpose of the research and the experimental protocols.

For enzyme-linked immunosorbent assay (ELISA) and quantitative reverse transcription polymerase chain reaction (RT-PCR), primary human conjunctival epithelial cells (PHCjECs) harvested from the conjunctival tissue obtained at the time of conjunctivochalasis surgery were cultured using a previously described method.[1] Briefly, conjunctival tissues were washed and immersed for 1 h at 37°C in 1.2 U mL^{-1} purified dispase (Roche Diagnostic Ltd., Basel, Switzerland). Then, the epithelial cells were detached, collected, and cultured in a low-calcium defined keratinocyte-SFM medium with defined growth-promoting additives (Invitrogen, Carlsbad, CA), including insulin, the epidermal growth factor, fibroblast growth factor, and a 1% antibiotic–antimycotic solution. Cell colonies usually became visible within 3 to 4 days. After reaching 80% confluence in 7–10 days, the cultured PHCjECs were used in subsequent procedures.

Enzyme-linked immunosorbent assay

We performed ELISA to confirm protein production. The amount of CXCL10, CXCL11, RANTES, MCP-1, and IL-6 released into the culture supernatant was determined by ELISA using the human CXCL10, CXCL11, RANTES DuoSet (R&D Systems, Inc., Minneapolis, MN) or the OptEIA™ MCP-1, IL-6 set (BD Pharmingen, San Diego, CA), respectively, in accordance with the manufacturer's instructions.[1]

Quantitative RT-PCR

Total RNA was isolated from PHCjECs using the RNeasy Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. For the RT reaction, we used the SuperScript™ Preamplification kit (Invitrogen). Quantitative RT-PCR was on an ABI-prism 7700 (Applied Biosystems, Foster City, CA) instrument using a previously described protocol[1] and the manufacturer's instructions. The primers and probes for CXCL10, CXCL11, RANTES, MCP-1, IL-6, and human GAPDH were purchased from Applied Biosystems. To amplify cDNA, PCR was performed in a 25-μL total volume that contained a 1 μL cDNA template in 2×TaqMan universal PCR master mix (Applied Biosystems) at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The results were analyzed with sequence detection software (Applied Biosystems). The quantification data were normalized to the expression of the housekeeping gene GAPDH.

Murine EAC

Balb/c mice were purchased from CLEA (Tokyo, Japan) and sensitized at 6–12 weeks of age. They were maintained on a 12-h light/12-h dark cycle under specific pathogen-free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto Prefectural University of Medicine. All studies were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Using our murine EAC model[6] on day 0, the mice were immunized with a subcutaneous injection into their left hind footpads of ragweed (RW) adsorbed on alum (200 μg RW and 2.6 mg alum in a total volume of 200 μL). On day 7, they received an intraperitoneal injection of RW adsorbed on alum and on day 18, their eyes were challenged with RW in PBS (500 μg in 5 μL per eye) or PBS alone (controls, 5 μL per eye). We administered rebamipide eyedrops (0.6%, 2%, or 6% rebamipide) 1 h before and 2-, 5-, and 8 h after short RW pollen challenge on the day of antigen challenge. Eyes were collected 24 h postchallenge for a histologic study.

Histological analysis

Using our previously described method,[6] we dissected whole eyeballs together with the eyelids and conjunctiva. This was followed by fixation in 10% neutral buffered formalin and embedding in paraffin blocks. Vertical 6-μm-thick sections were affixed to microscope slides, deparaffinized, and stained with the Luna's method. Stained eosinophils were counted under a light microscope. The number of eosinophils infiltrating the lamina propria mucosae of the tarsal of the conjunctiva in the entire section was recorded. We used sections from the central portion of the eye; they included the pupil and optic nerve head. Since the cell number varied according to the area counted, cell-count data are expressed as the number of infiltrating eosinophils divided by the area of the count (mm^2) measured by Scion Image software (Scion Corp., Frederick, MD). Data are presented as the mean ± SEM of all examined mice.

Compounds and reagents

Rebamipide was supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). RW, RW extract, and aluminum hydroxide (alum) were purchased from Polysciences, Inc. (Warrington, PA), LSL Co., Ltd. (Tokyo, Japan), and Sigma (St. Louis, MO), respectively.

Data analysis

Data were expressed as the mean ± SEM and evaluated by Student's *t*-test using the Microsoft Excel software program.

Results

Rebamipide downregulated the production of cytokines induced by polyI:C stimulation

As many transcripts in PHCjECs are significantly upregulated upon polyI:C stimulation,[1] we first examined whether rebamipide downregulated the polyI:C-induced production of cytokines in these cells. We performed ELISA to assess the effects of rebamipide on the polyI:C-induced production of CXCL10, CXCL11, RANTES, MCP-1, and IL-6. To determine the optimal dose, we administered rebamipide at doses of 2-, 0.2-, and 0.02 mM. As polyI:C-induced cytokine production was suppressed dose dependently (data not shown), we concluded that the optimal dose was 2 mM.

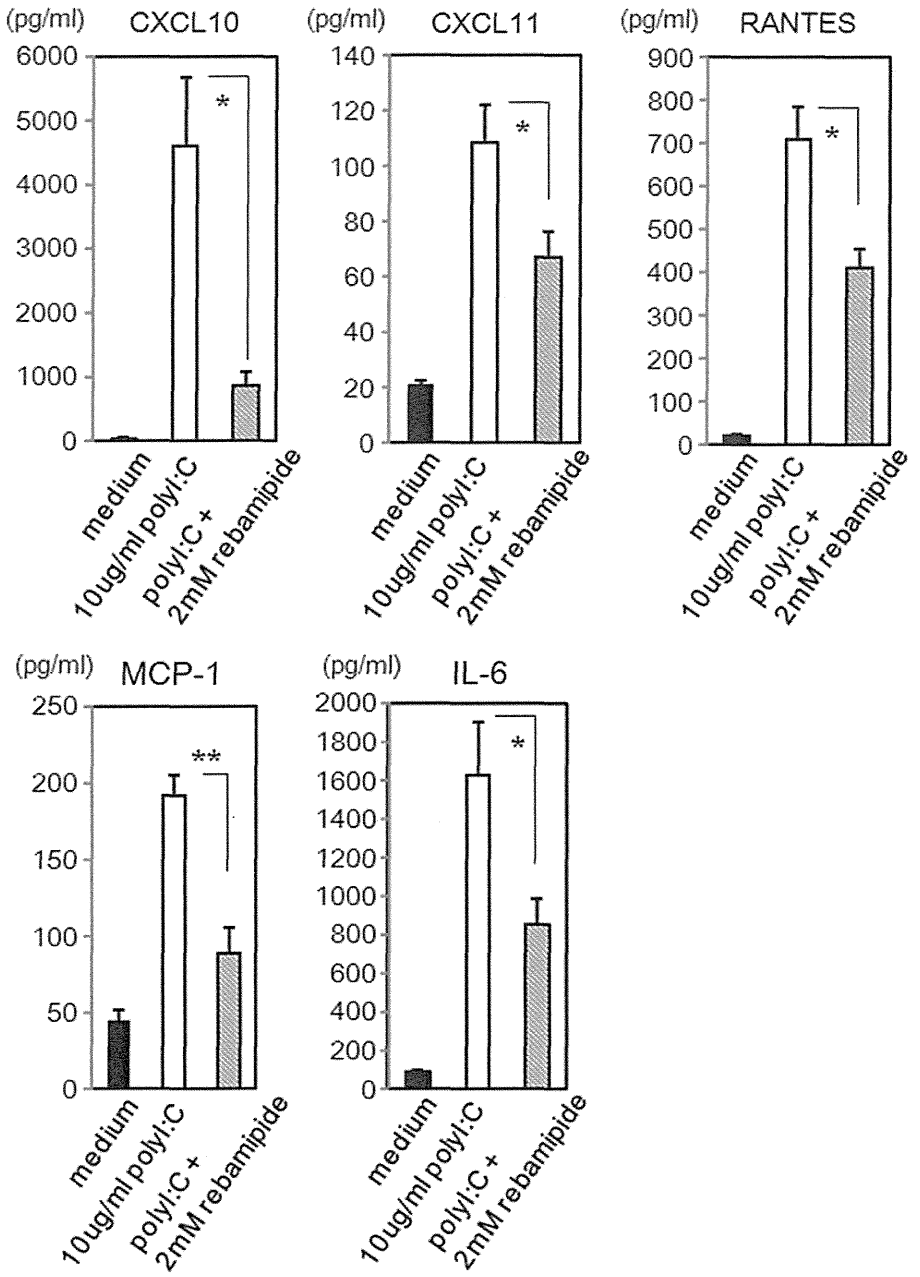

FIG. 1. *Effect of rebamipide on polyI:C-induced cytokine production.* PHCjEC were exposed to 10 μg/mL polyI:C and 2 mM rebamipide for 24 h. Data are representative of 3 separate experiments and are given as the mean ± SEM from one experiment carried out in 6 wells per group. (*$P < 0.05$, **$P < 0.005$.)

PHCjECs were therefore exposed to 10 μg/mL polyI:C and 2 mM rebamipide for 24 h. We found that rebamipide significantly attenuated the production of CXCL10, CXCL11, RANTES, MCP-1, and IL-6 (Fig. 1). We also examined the polyI:C-induced production of IL-8, however, it could not be downregulated by rebamipide (data not shown).

Rebamipide downregulated the mRNA level of cytokines induced by polyI:C stimulation

We performed quantitative RT-PCR to examine the effects of rebamipide on the polyI:C-induced expression of mRNAs for CXCL10, CXCL11, RANTES, MCP-1, and IL-6 in PHCjECs. After exposing the cells to 10 μg/mL polyI:C and 2 mM rebamipide for 6 h, the expression of mRNAs for these cytokines was significantly attenuated (Fig. 2).

Effect of rebamipide eye drops

We already knew that TLR3, a receptor of polyI:C, positively regulated allergic eosinophilic inflammation[5] and that rebamipide suppressed the polyI:C-induced production of cytokines, including allergy-related proteins such as RANTES in conjunctival epithelial cells *in vitro*. Therefore, we investigated whether the allergic eosinophilic inflammation in EAC could be suppressed by rebamipide eyedrops. On the day of challenge, we administered rebamipide eyedrops (0.6%, 2%, or 6% rebamipide per 5 μL) at 4 different time points to the eyes of RW-sensitized wild-type mice. We found that the infiltration of eosinophils was suppressed in a dose-dependent manner. At concentrations of 2% and 6%, the antigen-induced infiltration of eosinophils was significantly inhibited compared to the vehicle-treated controls

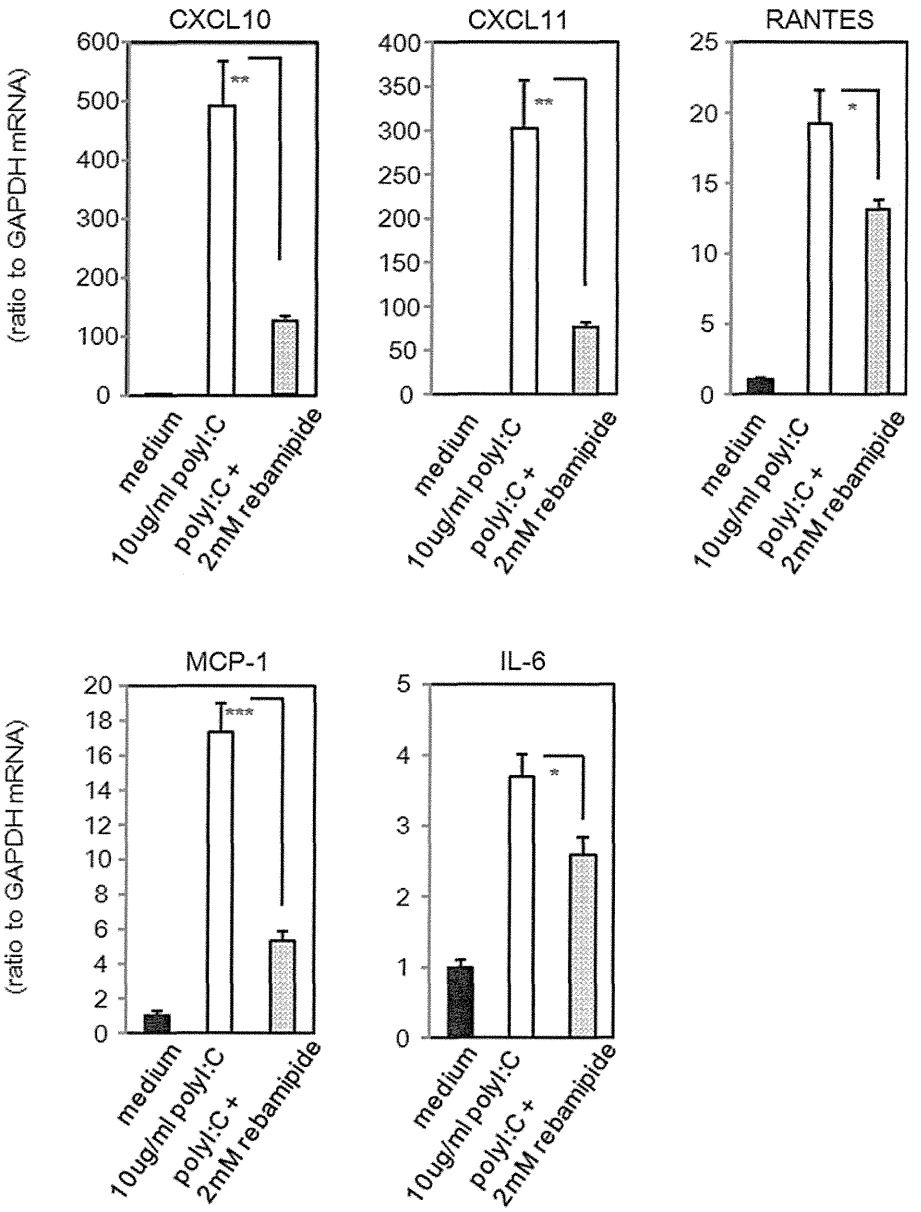

FIG. 2. *Effect of rebamipide on the polyI:C-induced mRNA expression of cytokines.* PHCjEC were exposed to 10 μg/mL polyI:C and 2 mM rebamipide for 6 h. The quantification data were normalized to the expression of the housekeeping gene *GAPDH*. The *y* axis shows the increase in specific mRNA over unstimulated samples. Data are representative of 3 separate experiments and are given as the mean ± SEM from one experiment carried out in 9 wells per group. (*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$.)

(Fig. 3 and Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/jop).

Discussion

Elsewhere we showed that ocular surface epithelial cells could regulate ocular surface inflammation[1–4,13–20] and posited that TLR3 upregulates, while EP3, a PGE_2 receptor, downregulates ocular surface inflammation.[5–7]

Here we demonstrate that in human conjunctival epithelial cells, rebamipide suppressed the polyI:C-induced production and expression of mRNAs for cytokines, and that its topical administration suppressed allergic eosinophil infiltration in our EAC model. These findings suggest that the topical delivery of rebamipide to the ocular surface might suppress ocular surface inflammation.

Rebamipide, a gastroprotective drug, has been used to treat gastric ulcers and gastritis. In rats, the drug accelerated the healing of acetic acid-induced gastric ulcers.[21] Although the precise mechanisms of action remain unknown, its beneficial effect on the gastric mucosa has been variously attributed to increased gastric mucus production,[8,9] suppression of gastric mucosal inflammation,[10,11] inhibition of neutrophil activation,[22,23] and hydroxylradical scavenging.[22,24]

Naito et al. administered rebamipide tablets (100 mg) to 32 patients with chronic gastritis. They were then subjected to gastroscopy and the rebamipide level in the gastric mucosa and in serum from venous blood was assayed. Between 30- and 120-min postingestion, the mean mucosal concentration of rebamipide was 60.0 ± 109.8 μg/g tissue, higher than 0.1 mM (37 μg/mL), and its mean serum concentration was 0.25 ± 0.23 μg/mL, lower than 1.0 μM (0.37 μg/mL). Their findings indicate that the concentration of rebamipide in the gastric mucosa was attributable to local penetration, suggesting that its blood level and systemic distribution play only a minor role in its antioxidative and antineutrophilic activities and that rebamipide acts directly on the gastric mucosa.[12]

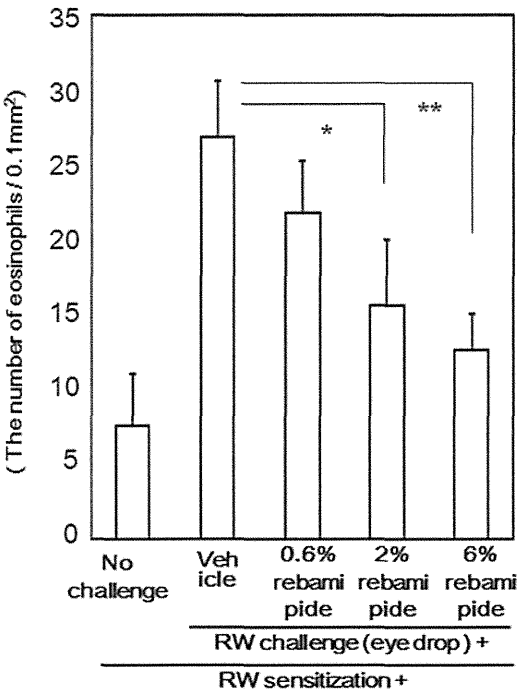

FIG. 3. Effect of rebamipide on conjunctival allergic eosinophilic infiltration in murine experimental allergic conjunctivitis. The number of eosinophils in the lamina propria mucosae of the tarsal conjunctiva was quantified. The data are shown as the mean ± SEM of samples from all examined mice (vehicle: $n = 16$, 0.6% rebamipide: $n = 16$, 2% rebamipide: $n = 21$, 6% rebamipide: $n = 14$) $^*P < 0.05$, $^{**}P < 0.005$.

In Japan, rebamipide eyedrops are used to treat dry eyes, because it could increase the level of mucin-like substances and improve the corneal and conjunctival epithelial damage. Urashima et al.[25] investigated the effects of rebamipide on the number of periodic acid Schiff (PAS)-positive cells in the conjunctiva, the mucin content in the cornea and conjunctiva of normal rabbits, and the effects of rebamipide on the desiccation-induced corneal damage. They found that it increased the number of PAS-positive cells in the conjunctiva and increased the amount of mucin-like substances in the conjunctiva and cornea. It also lowered the rose bengal scores of the cornea in their desiccation-induced corneal damage model. They proposed rebamipide as a candidate drug for the treatment of human corneal and conjunctival epithelial damage because it acts to increase the level of mucin-like substances.

Based on findings suggesting that rebamipide affects the mucosa directly, we considered that it may also have effects on ocular surface epithelial cells and that it may suppress ocular surface inflammation. We found that rebamipide did suppress the polyI:C-induced production of cytokines in conjunctival epithelial cells and that its topical administration suppressed allergic eosinophil infiltration in our EAC model. Therefore, we suspect that the topical application of rebamipide to the human ocular surface may suppress ocular surface inflammation.

Our observations suggest that the use of rebamipide may open new strategies for treating human ocular surface inflammation, including allergic conjunctivitis and dry eye diseases by modifying epithelial cell functions. However, the mechanisms underlying the anti-inflammatory and anti-allergic effects of rebamipide remain to be elucidated because the receptor for rebamipide remains unknown. It was reported that rebamipide increased PG levels, including PGE_2 on gastric tissue.[26] We reported that PGE_2 could also downregulate the production of polyI:C-induced cytokines in human ocular surface epithelial cells.[3] Intriguingly, IL-8 production induced with polyI:C could not be downregulated by rebamipide as same as by PGE_2, although the suppression effect by rebamipide of the 5 cytokine production (CXCL10, CXCL11, RANTES, MCP-1, and IL-6) might be smaller than by PGE_2. Therefore, it is possible that rebamipide might exert the anti-inflammatory effects through PGE_2. On the other hand, it is also possible that rebamipide might suppress the inflammation induced by not only TLR3, but also other mechanisms.

Investigations are underway in our laboratory to identify the precise molecular mechanisms of its anti-inflammatory and antiallergic effects because their identification may help to define the mechanisms of regulation of inflammation involving epithelial cells.

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Financial Relationship with Manufacturer

We declare that the work described in the present article was carried out in collaboration with Otsuka Pharmaceutical Co., Ltd., who supplied rebamipide used in this study.

Authors' Contributions

Material contributions to the research: Mayumi Ueta, Chie Sotosono, Norihiko Yokoi, and Shigeru Kinoshita; Writing and review contributions to the manuscript: Mayumi Ueta

Author Disclosure Statement

No competing financial interests exist.

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REVIEW ARTICLE

Long-term outcome of patients with severe cutaneous adverse reactions

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ABSTRACT

Visceral involvement associated with Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is well documented. However, little is known about the long-term outcomes of severe drug eruptions due to a lack of long-term follow-up. Long-term sequelae may arise in patients who survive the acute complications of severe drug reactions. In SJS/TEN, extensive scarring that result from the healing of mucocutaneous ulcerative lesions may interfere with organ function. Severe sequelae include visual impairment and pulmonary obliterative disease that impair patients' quality of life. In DIHS/DRESS, recent observations suggest that fulminant type 1 diabetes mellitus (FT1D) and autoimmune diseases such as autoimmune thyroiditis and lupus erythematosus can occur after a disease-free period of several months to years. Thus, DIHS/DRESS may lead to the development of autoimmune diseases, which may be overlooked. Dermatologists need to be aware of the sequelae that may arise following resolution of severe cutaneous adverse reactions and should be vigilant for manifestations of autoimmune disease during follow-up.

Introduction

Severe cutaneous adverse eruptions include Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). Visceral involvement can occur during the course of these diseases, including sepsis and pneumonitis in SJS/TEN, and hepatitis and renal failure in DIHS/DRESS. However, the long-term sequelae after resolution of severe drug eruptions is not well known, due to the lack of long-term follow-up and the potential development of sequelae after a disease-free period of several months to years. As there is limited information on the long-term outcomes of severe drug eruptions,[1–3] we present a review on the long-term outcomes of SJS/TEN and DIHS/DRESS.

Sequelae in SJS/TEN

SJS/TEN are rare, potentially life-threatening conditions triggered by drug administration and infections.[4,5] SJS and TEN are now recognized as variants of the same condition with differing severities. Although the pathomechanism of epidermal necrosis in SJS/TEN remains unknown, various factors have been implicated, including drug-specific T cells and/or monocytes/macrophages,[6] regulatory T cell function,[7,8] Fas/Fas ligand and perforin/granzyme B,[9] pro-inflammatory cytokines,[10,11] and granulysin produced by natural killer cells.[12] The skin and mucous membrane are affected in SJS/TEN, and mucosal involvement can be more severe than cutaneous involvement. Healing of ulcerative mucosal lesions may result in extensive scarring that interferes with organ function. Ocular and dermatologic long-term sequelae may occur and affect patients' quality of life, emphasizing the need for long-term follow-up of patients after resolution of SJS/TEN.

Ocular sequelae in SJS/TEN

Ocular sequelae in patients with SJS/TEN are well documented. The involvement of the ocular surface is very common and can result in long-term complications. In the acute disease, many patients experience mild to severe ocular involvement,[1] which include conjunctivitis and epithelial sloughing in mild cases, and pseudomembranous and membranous conjunctivitis and corneal and/or conjunctival epithelial defects with severe pain and photophobia in severe cases.[13] Inflammation of the ocular surface frequently persist

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after complete resolution of cutaneous lesions, leading to ocular sequelae in chronic stage, at least 1 year from the onset of SJS/TEN. Even minor involvement of the ocular surface in the acute disease may lead to chronic ocular discomfort that requires long-term therapy.

The ocular sequelae are broadly classified into three categories depending on the involving area. Corneal sequelae include superficial punctate keratinopathy, epithelial defect, loss of the palisades of Vogt, conjunctivalization, neovascularization, opacification, and keratinization. Conjunctival sequelae include conjunctival hyperemia and symblepharon formation. Eyelid sequelae include trichiasis, mucocutaneous junction involvement, meibomian gland involvement, lacrimal gland and duct involvements, and punctal damage (Figure 1).[1] These lesions can cause prolonged ocular discomfort and visual impairment, and may require long-term therapy. Among ophthalmic problems, severe dry eye is the most common long-term ocular sequela and is present in approximately 50% of patients with SJS/TEN. Trichiasis is also a common ocular sequela. In particular, corneal involvement such as neovascularization, opacification, and keratinization correlates with visual acuity (Table 1).

With respect to possible causes, *Mycoplasma pneumoniae*-associated SJS induced more ocular involvement during the acute stage than drug-induced SJS. *Mycoplasma pneumoniae*-associated SJS seldom caused long-term ocular sequelae in children, while adult patients remained at risk for long-term sequelae.[14,15] Recent genotype analyses revealed that multiplicative interactions of human leukocyte antigen (HLA)-A and Toll-like receptor 3 (TLR3) genes might be required for the development of ocular complications in SJS/TEN.[16]

Mucocutaneous sequelae in SJS/TEN

Despite documented involvement of the genitals in female patients with SJS/TEN, little information exists regarding long-term genital complications. Genital involvement during SJS/TEN includes erosive and ulcerative vaginitis, vulvar bullae, and vaginal synechiae.[17] Extensive scarring that affects genital function may occur with the healing of mucosal ulcerations.

Vaginal or vulvar areas of necrosis may form adhesions. Very few cases of symptomatic vaginal obstruction after SJS/TEN have been documented. Pathologic changes in the vulvovaginal area have been observed in women with SJS/TEN. Vulvovaginal adenosis/endometriosis—defined by the presence of metaplastic cervical or endometrial glandular epithelium within the vaginal wall—has been reported, causing dyspareunia and postcoital bleeding.[17] The cause remains unknown; it has been proposed that tubal or uterine epithelium implant over the raw areas during SJS/TEN.[18] The malignant potential of adenosis is unknown, but transformation to adenosis with cellular atypia of the vagina has been reported.[17] In a pediatric case, extensive labial synechiae and hydrocolpos occurred several years after an episode of SJS/TEN. Amenorrhea, cyclical abdominal pain, or a hypogastric mass in girls after an episode of SJS/TEN may indicate acquired vaginal obstruction. Thus, after a diagnosis of SJS/TEN in girls, it is prudent to schedule a prepubertal genital examination to avoid obstructed menstruation and future sexual problems.[18]

Several strategies to prevent vulvovaginal sequelae have been described. The application of intravaginal glucocorticoids, use of vaginal molds, and menstrual suppression during SJS/TEN have been proposed to reduce the formation of adhesions and limit metaplastic changes in affected areas.[17]

Other mucosal sequelae resulting from an obstructed urinary system include urinary retention and recurrent cystitis. Persistent lingual ulcerations and recurrent oral aphthae can be observed months after the resolution of SJS/TEN.[19]

Unlike mucous membranes, the skin usually heals within weeks without scarring if wounds are treated adequately. The development of hypertrophic scars in SJS/TEN has rarely been described in the literature.[20] Other cutaneous sequelae include dyspigmentation, nail deformity and fingernail loss, and xeroderma. It is likely that nail involvement is associated with ophthalmic involvement (Figure 2).[21] It is well known that Sjögren-like syndrome frequently

Table 1 Sequelae in SJS/TEN.

Ocular lesion
Cornea: superficial punctate keratinopathy, epithelial defect, loss of palisades of Vogt, conjunctivalization, neovascularization, opacification, keratinization
Conjunctiva: conjunctival hyperemia, symblepharon formation
Eyelid: trichiasis, mucocutaneous junction involvement, meibomian gland involvement, lacrimal gland and duct involvements, punctal damage
Mucocutaneous lesion
Urogenital system: vaginal obstruction/vaginal stenosis, vulvovaginal adenosis/endometriosis, urinary stream egress obstruction
Skin: pigmentary change, dry skin (xeroderma), appearance of melanocytic nevi or ectopic sebaceous gland, nail deformity
Pulmonary lesion
Obliterative bronchitis/bronchiolitis
Esophageal lesion
Stricture formation

SJS/TEN = Stevens-Johnson syndrome/toxic epidermal necrolysis.

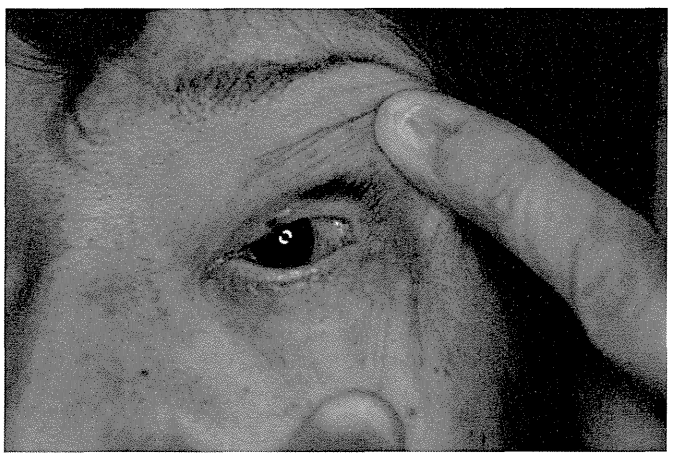

Figure 1 Blepharosynechia after resolution of toxic epidermal necrolysis (TEN).

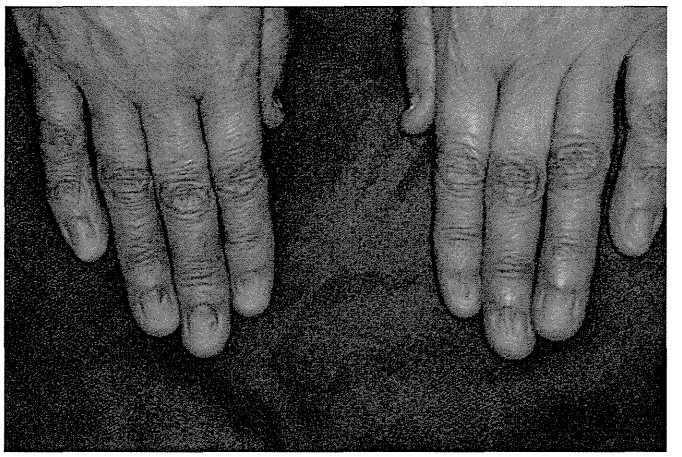

Figure 2 Nail loss after resolution of toxic epidermal necrolysis (TEN).