locally at injection sites or systemically on occasion, and combination therapy with RBV has been reported to cause an increased incidence of cutaneous reactions. These reactions include generalized pruritus, xerosis, erythematous papules and microvesicles mainly localized to the limbs and areas of friction.[10–14] Usually, topical corticosteroids or emollients are effective in managing the cutaneous reactions, and discontinuation of the antiviral drugs is not required.[10]

Because dermatological adverse reactions have been observed in telaprevir monotherapy and PEG-IFN and RBV combination therapy, there is a possibility that dermatological reactions develop even more frequently in the triple therapy. Phase II trials in the USA and in European countries (EU) show a higher incidence of dermatological adverse reactions in the telaprevir, PEG-IFN and RBV group than in the PEG-IFN and RBV group.[2,3] On the basis of this finding, for the Japanese phase III trials, we classified the severity in the same manner as that of the US/EU trials (Table 1) and collected detailed information. We hereby show the characteristics of the dermatological adverse reactions of telaprevir-based therapy, and consider the criteria for drug discontinuation and management plan for these reactions.

METHODS

Patients

Multicenter, randomized, phase III trials were performed at 42 Japanese medical institutions from 2008 to 2010. In these trials, 126 TN patients and 141 treatment-failure (TF) patients were administrated telaprevir, PEG-IFN and RBV for 12 weeks followed by PEG-IFN and RBV for another 12 weeks (T12/PR24 group, $n = 267$), and 63 TN patients were administrated PEG-IFN and RBV for 48 weeks (PR48 group).[6,7] The TF patients comprised 109 relapsers and 32 non-responders.[7] The principal eligibility criteria were as follows: (i) a diagnosis of CHC; (ii) infection with HCV-1; (iii) HCV RNA levels of 5.0 $\log_{10}$ IU/mL or more; (iv) age at entry, 20–65 years; and (v) bodyweight of more than 40 kg and 120 kg or less. The main exclusion criteria were as follows: (i) hemoglobin level of less than 12 g/dL, neutrophil count of less than 1500/mm^3 and platelet count of less than 100 000/mm^3; (ii) positive for antibodies against hepatitis B surface antigen or HIV; and (iii) chronic renal failure or a creatinine clearance of 50 mL/min or less.

Each patient gave a written informed consent before participating in these studies.

Study design

The clinical trial involving TN patients was a multicenter, randomized, controlled study, where 189 patients were assigned to either the T12/PR24 group or the PR48 group.[6] The trials that included TF patients (relapsers and non-responders) were open-label studies that included the T12/PR24 group alone.[7]

For patients in the T12/PR24 group, telaprevir was administrated p.o. t.i.d. every 8 h at a dose of 750 mg after meals for 12 weeks. PEG-IFN-α-2b (PegIntron®; MSD, Tokyo, Japan) was administrated s.c. once a week (1.5 μg/kg; range, 1.250–1.739 μg/kg) and RBV (Rebetol®; MSD) was administrated p.o.

Table 1. Severity classification of dermatological adverse reactions in phase III trials in Japan

Severity	Criteria	Management
Grade 1	Involvement of ≤50% of the body surface, localized No evidence of systemic symptoms	Consultation with a dermatologist, if needed
Grade 2	Involvement of ≤50% of the body surface, multiple or diffuse lesions Or rash with any of the following characteristics: Mild systemic symptoms Mucous membranes involved but with no ulceration/erosion	Discontinuation of the study drugs is generally not necessary, the investigators can consider the following, if needed: Discontinuation of telaprevir Discontinuation, interruption, or reduction of PEG-IFN and RBV
Grade 3	Generalized rash involving >50% of the body surface Or rash with any of the following characteristics: Appearance of significant systemic symptoms that are new and are considered to be related to the onset and/or progression of the rash Ulceration/erosion of mucous membranes Epidermal detachment (epidermal necrosis or separation of epidermis from underlying dermis) Target lesions Vesicles or bullae Palpable purpura	Consultation with a dermatologist Discontinuation of telaprevir (in principle) Consider discontinuation or reduction of PEG-IFN and RBV
Life-threatening	SJS, TEN, DRESS,† EM‡ Or other life-threatening symptoms, Or cases where features of serious disease are observed	Consultation with a dermatologist Immediate discontinuation of all drugs

†DRESS corresponds to the Japanese term for DIHS.
‡EM is not life-threatening, but in severe cases, mucous membrane lesions and systemic symptoms are encountered. In phase III trials, EM was reported in five patients and was serious in one patient. DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; EM, erythema multiforme; PEG-IFN, pegylated interferon; RBV, ribavirin; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

b.i.d. after meals (200–600 mg/dose; daily dose, 600–1000 mg) for 24 weeks. The patients in the PR48 group received PEG-IFN-α-2b and RBV at the aforementioned doses for 48 weeks. The doses of PEG-IFN-α-2b and RBV were reduced when the patients' hemoglobin level, white blood cell count, neutrophil count or platelet count decreased, or when adverse events developed.[6,7] Patients in the T12/PR24 group and PR48 group were followed up for 24 weeks.

Assessment

Dermatological adverse reactions were investigated during the administration period of 24 weeks in the T12/PR24 group and 48 weeks in the PR48 group, and during the 24-week follow-up period in both groups. We integrated the data of the 126 TN patients and 141 TF patients (109 relapsers and 32 non-responders) who were administrated telaprevir, PEG-IFN-α-2b and RBV in three phase III trials (Table 2).

Table 1 shows the severity classification system used to evaluate dermatological adverse reactions. When the skin reactions were detected, the investigators referred the patients to a dermatologist as needed, and reported the events in reference to the diagnosis of the dermatologist. In cases where the severity was considered as grade 3, telaprevir was discontinued in principle. In addition, in cases where severe cutaneous reactions including Stevens–Johnson syndrome (SJS) and drug rash with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS) were suspected, all study drugs were immediately discontinued.

Of the dermatological adverse reactions reported by investigators, we examined 355 events in the T12/PR24 group (190 events in 111 TN patients and 165 events in 115 TF patients), and 102 events in the PR48 group (among 53 TN patients). The events such as contact dermatitis and impetigo that were unlikely to be attributable to the study drugs were excluded (Table 2).

RESULTS

Study patients

In the T12/PR24 (TN) group, the T12/PR24 (TF) group, and the PR48 group, the number of men was 66 (52.4%), 83 (58.9%), and 33 (52.4%), respectively; the median age was 53 (range: 20–65), 57 (range: 20–65), and 55 (range: 20–65) years, respectively; and the median body mass index was 22.55 (range: 16.2–31.1), 23.00 (range: 17.1–32.4), and 23.30 (range: 17.9–30.8) kg/m^2, respectively.

Incidences of dermatological adverse reactions

Table 3 summarizes the incidences of dermatological adverse reactions. The incidence of localized or systemic rash-related events, excluding reactions on the injection site only, was higher in the T12/PR24 groups than in the PR48 group, whereas events limited to the injection site were more common in the latter. The incidences of grade 2 and grade 3 rash-related events were also higher in the T12/PR24 groups than in the PR48 group. No statistical difference was observed in the incidence of rash-related events between the T12/PR24(TN) and T12/PR24(TF) groups.

Table 4 shows the rates of serious adverse events, discontinuation, interruption, and reduction of study drugs due to the dermatological events. No dermatological problem led to death. Serious adverse events requiring hospitalization or prolongation of inpatient care were observed in only the T12/PR24 (TN) and T12/PR24 (TF) groups, respectively. Two patients developed SJS and one developed DRESS/DIHS.

Because no clear differences were found in incidences, grades and features of dermatological adverse reactions between TN and TF patients in the T12/PR24 group, we integrated the data of these two patient groups to evaluate the reactions associated with triple therapy as described below.

Time to onset

Figure 1 shows the cumulative incidence of dermatological adverse reactions obtained using the Kaplan–Meier method. The incidence up to week 4 was 77.1% in the T12/PR24 groups and 55.6% in the PR48 group. Time to onset of the first reaction was earlier in the T12/PR24 group than in the PR48 group (Fig. 1a).

Most grade 1 events occurred within 2 weeks in the T12/PR24 group, and were approximately the same timing as those in the PR48 group. Grade 2 events occurred early in the T12/PR24 group, with increased incidence during weeks 4–8 after the initial administration (Fig. 1b). Regarding grade 3 events, papuloerythematous rashes affecting more than 50% of the body surface area occurred within 1 week, and rashes with

Table 2. Number of patients and dermatological adverse events in each study

		T12/PR24 group			PR48 group		
		Dermatological reactions			Dermatological reactions		
		n	No. of patients	No. of events	*n*	No. of patients	No. of events
Study on treatment-naïve patients	–	126	111	190	63	53	102
Studies on treatment-failure patients	Relapser	109	85	124	–	–	–
	Non-responder	32	30	41	–	–	–
	Total	141	115	165	–	–	–

T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Table 3. Incidence of dermatological adverse reactions

	T12/PR24						PR48	
	Treatment-naïve *n* = 126		Treatment-failure *n* = 141		Total *n* = 267		Treatment-naïve *n* = 63	
Total	111	88.1%	115	81.6%	226	84.6%	53	84.1%
Rash-related events[†]	94	74.6%	106	75.2%	200	74.9%	37	58.7%
Grade 1	53	42.1%	63	44.7%	116	43.4%	31	49.2%
Grade 2	43	34.1%	47	33.3%	90	33.7%	12	19.0%
Grade 3[§]	15	11.9%	9	6.4%	24	9.0%	3	4.8%
Injection site-related events[‡]	60	47.6%	36	25.5%	96	36.0%	37	58.7%
Grade 1	59	46.8%	36	25.5%	95	35.6%	37	58.7%
Grade 2	1	0.8%	0	–	1	0.4%	0	–
Grade 3	0	–	0	–	0	–	0	–

[†]Localized or systemic rash-related events, excluding reactions on the injection site only. The terms reported more than 10% were rashes (T12/PR24 vs PR48: 38.6% vs 28.6%), drug eruptions (26.6% vs 3.2%) and erythema (6.0% vs 20.6%).
[‡]The terms reported more than 10% were injection site erythema (T12/PR24 vs PR48: 19.1% vs 33.3%) and injection site reaction (16.1% vs 25.4%).
[§]Including life-threatening events, two Stevens–Johnson syndrome (SJS) cases, and one drug rash with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome case. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Table 4. Incidence of serious events and rates of discontinuation, interruption and reduction of study drug(s) due to dermatological events

	T12/PR24						PR48	
	Treatment-naïve *n* = 126		Treatment-failure *n* = 141		Total *n* = 267		Treatment-naïve *n* = 63	
Serious adverse events	3	2.4%	5	3.5%	8	3.0%	0	–
Discontinuation of any study drug	12	9.5%	11	7.8%	23	8.6%	2	3.2%
Telaprevir only	4	3.2%	7	5.0%	11	4.1%	–	–
All study drug(s)	8	6.3%	4	2.8%	12	4.5%	2	3.2%
Interruption of PEG-IFN or RBV	0	–	2	1.4%	2	0.7%	0	–
Reduction of PEG-IFN or RBV	2	1.6%	2	1.4%	4	1.5%	0	–

T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

pyrexia and lymphadenopathy, such as SJS and DRESS/DIHS, occurred during weeks 4–8 in the T12/PR24 group.

Characteristics of dermatological adverse reactions

Table 5 shows the characteristics of the 355 events in the T12/PR24 group and 102 events in the PR48 group.

Area of dermatological adverse reactions on the body surface. In the T12/PR24 group, 27.9% of dermatological adverse reactions (multiple or diffuse reactions) affected 50% or less of the body surface, while 5.4% affected more than 50% of the body surface. Compared with the PR48 group, the involved areas tended to be large in the T12/PR24 group.

Distribution. The dermatological reactions appeared mainly in the extremities and the trunk, and sometimes on the face. In case of SJS, DRESS/DIHS and serious erythema multiforme (EM), the intraoral areas, lips and pharynx were affected.

Features of lesions. Most of the dermatological adverse reactions in the T12/PR24 groups were papuloerythematous or maculopapular rashes (Figs 2a,b), which were similarly observed in the PR48 group; a few cases were judged as EM. In some cases, florid rashes developed at the injection site (Fig. 2c). In the SJS and DRESS/DIHS cases, superficial ulceration and erosion of mucous membranes or epidermal detachment were also observed.

Pruritus. Most dermatological adverse reactions were accompanied by pruritus in both the T12/PR24 group (82.5%) and the PR48 group (82.4%) (Table 5).

Systemic symptoms. In the T12/PR24 group, 7.0% of dermatological adverse reactions were accompanied by systemic symptoms, and pyrexia occurred most frequently. In the SJS, DRESS/DIHS and serious EM cases, pyrexia of 38–39°C and lymphadenopathy were observed (Table 5).

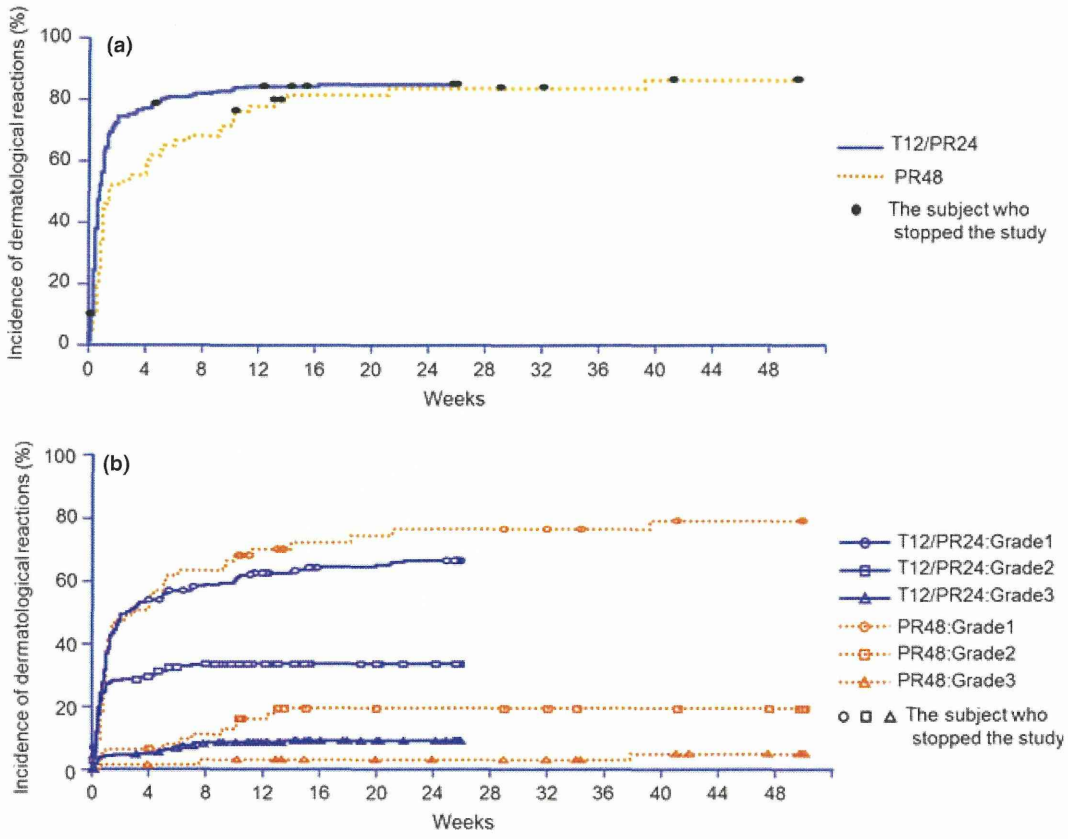

Figure 1. Time to onset of first dermatological adverse reaction. Cumulative incidence of dermatological adverse reactions (a) in total and (b) by grade in the T12/PR24 and PR48 groups obtained using the Kaplan–Meier method. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Serious cases

SJS. Stevens–Johnson syndrome was reported in two patients (0.7%) in the T12/PR24 group.

In one patient (female, 50 years old), a grade 1 rash was observed 8 days after the initiation of drug administration and it resolved 4 days later. On day 35, another rash appeared, and on day 44, when the patient was hospitalized, erythema affecting approximately 30% of the body surface with erosion of the intraoral mucous membrane was observed. The maximum body temperature was 39.3°C. All antiviral drugs were discontinued, and the patient was treated with systemic corticosteroids. Erythema progressed till 4 days after drug discontinuation (Fig. 2d) and resolved after 7 weeks.

In the other patient (female, 47 years old), "EM major" was diagnosed by a dermatologist but was integrated as SJS according to the clinical trial's coding rule. A localized rash was observed on day 3. Pyrexia up to 38.5°C and worsening of the rash were observed on day 25, and the patient was hospitalized due to erosion appearing on the lips and the mucous membrane of the pharynx. All study drugs were discontinued, and the patient recovered with the use of systemic corticosteroids 8 weeks after discontinuation.

DRESS/DIHS. In one patient (0.4%), a 60-year-old female, in the T12/PR24 group, DRESS/DIHS developed. Grade 1 rash was observed on day 7. On day 44, new redness appeared on the waist and legs, and on day 64 erythema worsened with persistent high fever; all study drugs were subsequently discontinued. The patient developed erythema with target lesions (Fig. 2e) and erosion of the oral mucous membranes on day 66. After discontinuation of the drugs, pyrexia over 38°C (maximum, 39.7°C) was observed. On the basis of symptom progression and laboratory test findings (increased white blood cell count [46300/μL], appearance of atypical lymphocytes [23.3%], raised eosinophil count [45.7%], high ferritin level, high lactate dehydrogenase level, lymphadenopathy and reactivation of human herpesvirus six based on a rise in titer from 1:160 [29 days after onset] to 1:2560 [57 days after onset]), a diagnosis of DRESS/DIHS was made. On administration of systemic corticosteroids, the patient recovered 11 weeks after discontinuation.

EM with mucous membrane lesions. Serious EM with mucous membrane lesions was observed in one patient (0.4%), a 58-year-old female, in the T12/PR24 group. On day 14, the patient developed a grade 1 rash, which resolved

Table 5. Characteristics of dermatological adverse reactions

	T12/PR24	PR48
No. of dermatological adverse reactions	355	102
Distribution area		
≤50% of body surface, localized	237 (66.8%)	87 (85.3%)
≤50% of body surface, multiple or diffuse	99 (27.9%)	15 (14.7%)
>50% of the body surface	19 (5.4%)	0 (0.0%)
Features of lesions[†]		
Erythema without target lesions	296 (83.4%)	89 (87.3%)
Erythema with target lesions	8 (2.3%)	0 (0.0%)
Purpura	22 (6.2%)	0 (0.0%)
Vesicles or bullae	7 (2.0%)	2 (2.0%)
Pustule	7 (2.0%)	0 (0.0%)
Ulceration or erosion of mucous membranes	4 (1.1%)	1 (1.0%)
Epidermal detachment	2 (0.6%)	1 (1.0%)
Other[‡]	85 (23.9%)	22 (21.6%)
Pruritus		
Yes	293 (82.5%)	84 (82.4%)
No	62 (17.5%)	18 (17.6%)
Systemic symptoms		
No	330 (93.0%)	102 (100.0%)
Yes[†]	25 (7.0%)	0 (0.0%)
Pyrexia	23 (6.5%)	0 (0.0%)
Angioedema	2 (0.6%)	0 (0.0%)
Lymphadenopathy	6 (1.7%)	0 (0.0%)
Other[§]	2 (0.6%)	0 (0.0%)

[†]Multiple features may co-exist.
[‡]For example, redness and papules.
[§]Inflammation of lips and dry skin. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

4 days later. The drug rash developed again on day 34, followed by pyrexia and enlargement of the rash on day 39, resulting in hospitalization of the patient. Pyrexia of up to 39.0°C was observed. Despite treatment with oral corticosteroids at 20 mg/day, the rash worsened and intraoral redness was observed 9 days after hospitalization; thus, all study drugs were discontinued. The patient's condition was diagnosed as EM, the dose of oral corticosteroids was increased to 60 mg/day and the patient recovered 11 weeks after drug discontinuation.

Treatment of dermatological adverse reactions

Figure 3 shows the medical treatments used for the dermatological events that developed during the antiviral administration period in the T12/PR24 group (347 events) and in the PR48 group (100 events). The main medical agents were topical corticosteroids and oral antihistamines in both groups. The strength of topical corticosteroids most often used were either class III (potent) or class IV (very potent).

Among the 347 dermatological events in the T12/PR24 group, 324 events did not require discontinuation of any of the study drugs. Approximately 21% events resolved without treatment. Events mainly treated with topical corticosteroids comprised 44.4%, and those treated with topical corticosteroids and oral antihistamines made up 17.9%. The frequency of treatment with systemic corticosteroids was only 4.6%. On the basis of these results, we considered almost all dermatological adverse reactions to be manageable with topical corticosteroids and oral antihistamines.

In the T12/PR24 group, 23 dermatological events required drug discontinuation. For these events, systemic corticosteroids were used more frequently (30.4%) in addition to topical corticosteroids and oral antihistamines.

Period of resolution

In the T12/PR24 group, 61.4% of the dermatological events resolved during the treatment period: 38.6% during the telaprevir treatment period and 22.8% during the PEG-IFN-α-2b and RBV treatment period. The proportion of dermatological events that resolved after completion or discontinuation of all the study drugs was 37.2% (Fig. 4).

In the PR48 group, 59.0% (59/100 events) of the dermatological events resolved by the end of the treatment period.

DISCUSSION

Compared to the dermatological adverse reactions associated with PEG-IFN and RBV therapy in phase III clinical trials, those caused by the telaprevir-based triple therapy: (i) developed early; (ii) affected large areas of the body; and (iii) were accompanied in several cases by severe rashes, mucous membrane lesions, epidermal detachment or systemic symptoms associated with high pyrexia or lymphadenopathy, including SJS and DRESS/DIHS. In addition, target lesions, purpura, and pustule were reported only in the triple therapy.

The dermatological adverse reactions associated with triple therapy were mostly of grade 1 or grade 2, and they were pruritic papuloerythematous lesions similar to the ones associated with PEG-IFN and RBV therapy. These adverse reactions could be managed with topical corticosteroids and oral antihistamines, and the associated discontinuation rate was low. Over 60% of the events resolved during the treatment phase. In view of these results and the therapeutic effect on CHC, early discontinuation of antiviral therapy due to mild or moderate dermatological adverse reactions should be avoided.

On the other hand, we cannot ignore the occurrences of SJS and DRESS/DIHS in the clinical trials. Aggravation of these serious events may lead to death and sequelae such as blindness. Therefore, on observation of clinical signs and symptoms that may lead to these serious conditions, all drugs must be discontinued immediately and appropriate treatment for these reactions should be administrated as soon as possible.

The criteria for drug discontinuation and treatment for dermatological reactions based on severity are illustrated in the algorithm in Figure 5. In addition to discontinuation of all drugs in cases of serious adverse reactions, a basic principle is to discontinue telaprevir in cases where the affected area exceeds 50% of the body surface (corresponding to grade 3) or where symptoms continued to worsen, notwithstanding a

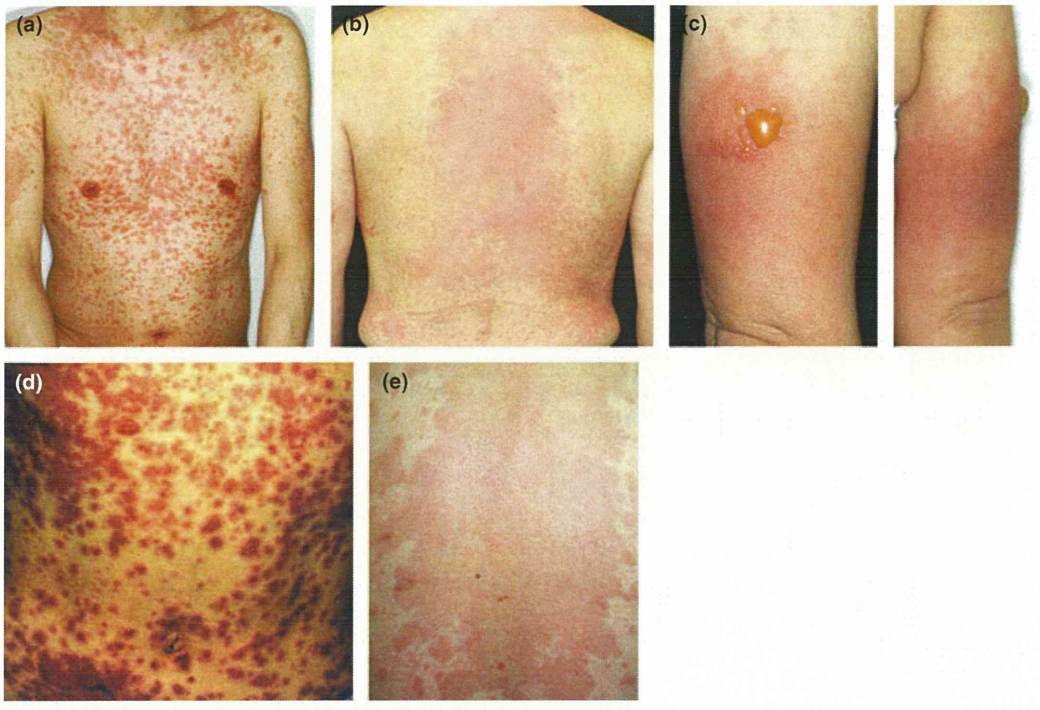

Figure 2. Examples of dermatological adverse reactions in the T12/PR24 group. (a,b) Grade 2 rashes, (c) injection site reaction, (d) Stevens–Johnson syndrome case, and (e) drug rash with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome case in the T12/PR24 group. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks.

grade 2 reaction. If symptoms worsen during the remaining period when only PEG-IFN and RBV are administrated, discontinuation of these two drugs is likewise recommended. Regarding the antiviral effects observed in the patients who discontinued any of the study drugs due to dermatological reactions, seven of the 11 patients who discontinued only telaprevir achieved SVR, and eight of the 12 patients who discontinued all study drugs achieved SVR.

Grade 1 and grade 2 reactions could be managed with topical corticosteroids and oral antihistamines. These medications were used for grade 3 reactions in a similar manner without any problems, although in some cases, such as SJS, DRESS/DIHS and other severe reactions, the systemic use of corticosteroids was required. In some patients, who continued to receive the study drugs concomitant with systemic corticosteroids, further aggravation of dermatological reactions was noted when the dose of corticosteroids was reduced or when their administration was discontinued. There is a possibility that use of systemic corticosteroids masked serious problems and the tapering of steroids aggravated reactions; hence, telaprevir discontinuation should be taken into consideration when systemic use of corticosteroids is required.

The cases of SJS, DRESS/DIHS and serious EM were accompanied by pyrexia of 38–39°C, erosions affecting the mucous membranes or conjunctival lesions. It is, therefore, important to detect early signs of serious diseases, and empowering patients to identify such symptoms by informing them about the features associated with these diseases, like pyrexia and mucous membrane erosion, will enable early detection.

The mechanism of telaprevir-related dermatological adverse reactions remains unknown. Because dermatological adverse reactions were observed even in the study on telaprevir monotherapy, it can be said that telaprevir per se is a factor for dermatological reactions. The dermatological adverse reactions in telaprevir monotherapy were, however, relatively milder than those in the telaprevir-based triple therapy. Therefore, it can be suggested that the concomitant administration of telaprevir with PEG-IFN and RBV led to an additive or synergistic effect on the adverse reactions and sometimes resulted in serious cases. In addition, in the triple therapy, the dermatological adverse reactions occurred within 1 week of administration in approximately half of the cases. As the onset time of these reactions was earlier than that of the usual drug rash (i.e. drug allergy), the mechanism underlying these early occurring reactions may be different from that of allergy in general.

In the US/EU clinical trials, pharmacokinetics, human leukocyte antigen genes and multidrug resistance 1 gene were examined to elucidate risk factor(s) for telaprevir-related dermatitis. However, no specific relation was noted between these factors and the development or severity of dermatological reactions.[15]

The incidence of skin adverse reactions (rash-related events) in phase II or phase III studies performed in the US/EU was 55–56% in the triple therapy group and 33–34% in the PEG-

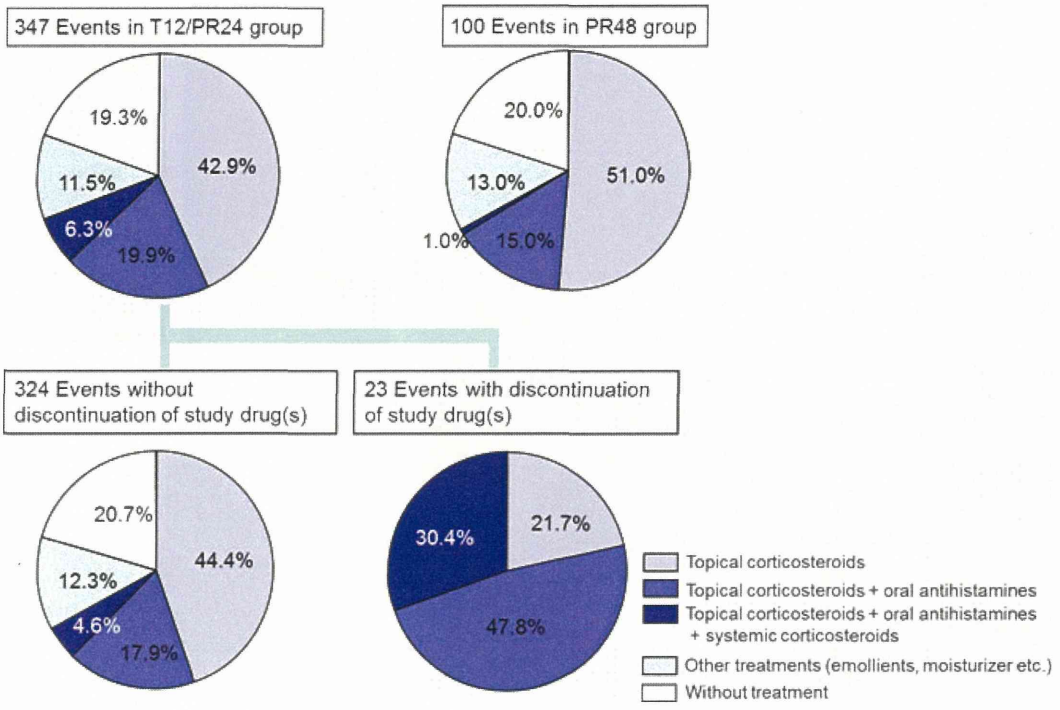

Figure 3. Treatment of dermatological adverse reactions. The medical agents shown were used for 347 events that developed during the antiviral administration period in the T12/PR24 group (324 events without drug discontinuation and 23 events with drug discontinuation) and for 100 events in the PR48 group. Treatment is classified according to the use of topical corticosteroids, oral antihistamines, systemic corticosteroids and others agents. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

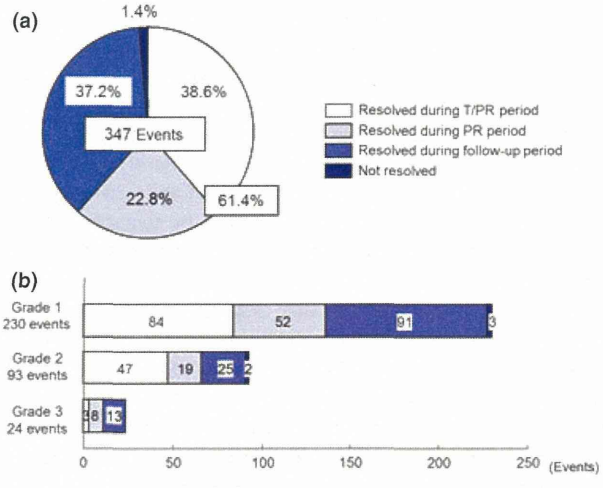

Figure 4. Period of resolution in the T12/PR24 group. (a) Of the 347 dermatological reactions that occurred during the treatment phase in the T12/PR24 group, approximately 61% resolved by the end of dosing. (b) The period of resolution of each dermatological adverse reaction is shown by grade. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks.

IFN and RBV therapy group; these rates are lower than those observed in Japanese patients (74.9% and 58.7%, respectively). In addition, 12–14% and 4–5% of the subjects developed grade 2 and grade 3 reactions, respectively, in the US/EU studies, while in Japan, the respective percentages were 33.7% and 9.0%.[15,16] Although the early onset and symptom features were common between the US/EU and Japan studies, there may be differences in the rate of symptom development and in severity worsening tendency. However, a stringent comparison is difficult between the US/EU and Japan studies, because there may be differences in the interpretation of severity classification systems including definition of the involved area or in the data collection and aggregation methods between these studies.

In conclusion, the telaprevir-based triple therapy often caused dermatological adverse reactions. Most reactions were of grade 1 or grade 2 and could be managed without discontinuation of the study drugs. Some patients, however, developed serious reactions such as SJS and DRESS/DIHS. What is most important is to consider the balance between the risk and benefit for an individual patient, and address individual reactions appropriately. For appropriate treatments of individual dermatological adverse reactions, the judgment of discontinuation of antiviral drugs and treatment based on the severity are extremely important in this triple therapy.

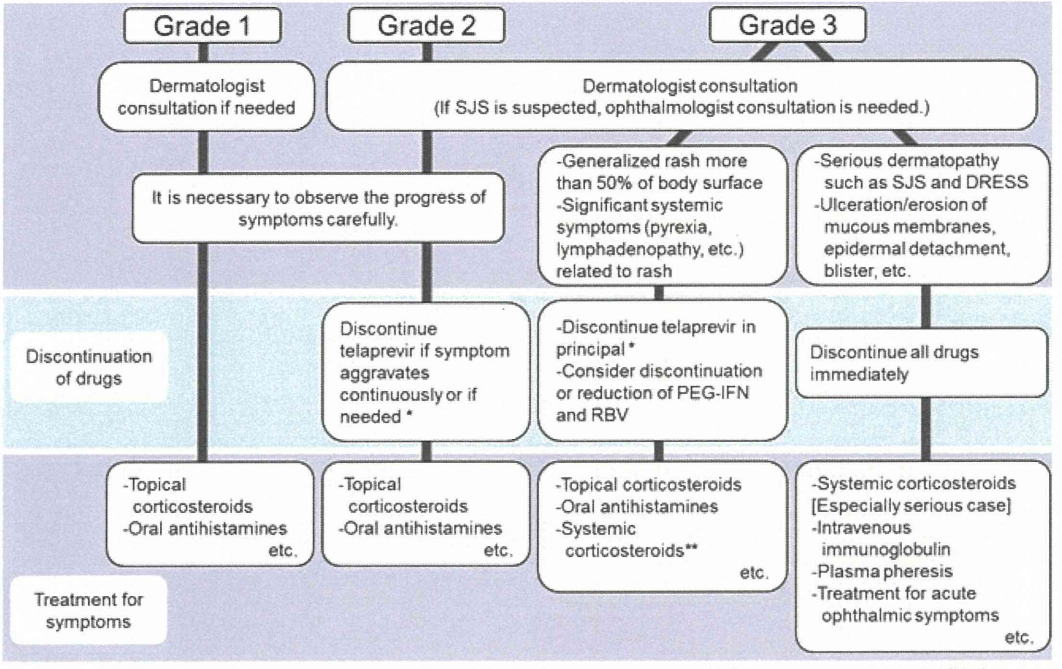

Figure 5. Algorithm for the discontinuation of drugs and treatment for dermatological adverse reactions.

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ORIGINAL ARTICLE

Distinguishing between erythema multiforme major and Stevens–Johnson syndrome/toxic epidermal necrolysis immunopathologically

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ABSTRACT

The early clinical presentations of Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are similar to that of erythema multiforme major (EMM). Cytotoxic molecules, especially granulysin, are expressed in the skin lesions of SJS/TEN and cause extensive keratinocyte death. It is postulated that the function of regulatory T cells (Treg) in SJS/TEN is inadequate. This study examined whether an immunohistological examination of cytotoxic molecules and the immunophenotype of Treg is useful for discriminating SJS from EMM in the early period. Over the past 9 years, the lesional skin of 14 patients with SJS/TEN and 16 patients with EMM was biopsied. Double immunofluorescence labeling of CD8 and granulysin, perforin, or granzyme B was performed, and immunohistochemical analyses of granulysin, perforin, granzyme B, CD1a, CD3, CD4, CD8, CD68 and Foxp3 were conducted using a highly sensitive indirect immunoperoxidase technique. The number of cells positive for each antibody per five high-power fields was counted. The proportions of granulysin$^+$ cells/CD8$^+$ cells (P = 0.012) and perforin$^+$ cells/CD8$^+$ cells (P = 0.037) in SJS/TEN were significantly higher than in EMM. The number of Foxp3$^+$ cells/five high-power fields in SJS/TEN was significantly lower than in EMM (P = 0.004). Similarly, the number of CD4$^+$ cells/five high-power fields in SJS/TEN was significantly lower than in EMM (P = 0.0017). These data suggest that these panels of antibodies for labeling cytotoxic molecules, CD4 and Treg are useful for discriminating early SJS/TEN and EMM with a skin biopsy.

Key words: cytotoxic molecules, Foxp3, granulysin, perforin, regulatory T cells.

INTRODUCTION

Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and erythema multiforme major (EMM) belong to the same disease spectrum.[1] SJS and TEN are now thought to be severity variants of the same disease.[2] However, SJS/TEN is quite different from EMM in terms of its associated mortality and the presence of severe sequelae, such as blindness, respiratory disturbance and scarring of the mucosa. There is general agreement that EMM with mucous membrane involvement and SJS/TEN are clinically different disorders.[2,3] Although the clinical presentation of SJS/TEN in the early phase is quite similar to that of EMM, a quick diagnosis is critical to determining the treatment.[4]

The specific signal molecules provoking the immune reaction to keratinocyte apoptosis in SJS/TEN are controversial. Recently, secretory granulysin was shown to be a key mediator of extensive keratinocyte death in SJS/TEN, whereas soluble Fas ligand, granzyme B and perforin were not.[5] The serum granulysin levels in most patients with SJS/TEN were elevated 2–4 days before skin detachment or eroded mucosal lesions developed;[6] therefore, monitoring the serum granulysin level enabled the early diagnosis of SJS/TEN.[7] However, the immunopathological localization of cytotoxic molecules in the skin lesions during the early phase of SJS/TEN has not been analyzed sufficiently.

A severe drug eruption could be provoked by imbalance of the immune system, such as the excessive activation of effector T cells and inadequate function of regulatory T cells (Treg).[8] In fact, impaired Treg function has been reported in TEN, and its role in severe epidermal damage was suggested.[8] In immunopathological analyses, there were fewer Treg cells in the skin lesions of TEN than in drug-induced hypersensitivity syndrome (DIHS).[8] However, no comparable data on the density of Treg between SJS/TEN and EMM have been reported.

This study examined whether immunohistological measures of cytotoxic molecules and the markers of Treg can discriminate between SJS/TEN and EMM in the early periods of these diseases.

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METHODS

Patients and specimens

Biopsy specimens from 10 patients with SJS, four patients with TEN and 16 patients with EMM seen over the past 9 years were collected retrospectively for this study. The final diagnosis of SJS/TEN in each case was based on the criteria of a research group with the Ministry of Health, Labor and Welfare of Japan, whereas that of EMM was based on the classification used by an international group of experts. In brief, EMM was defined as erythematous plaques with typical targets or raised atypical targets, and with neither extensive epidermal necrosis nor advanced mucous membrane involvement. The 14 patients with SJS/TEN were seven males and seven females with a mean age of 38.4 years (range, 1–68), and the 16 patients with EMM were eight males and eight females with a mean age of 44.4 years (range, 8–89). The number of days between the onset of the rash and the skin biopsy in SJS/TEN and EMM was 4.14 ± 3.48 and 4.88 ± 2.87, respectively. This study was approved by the ethics committee of our institution.

Immunohistochemistry

Immunohistochemistry was performed using a highly sensitive indirect immunoperoxidase technique. For antigen retrieval, sections were incubated for 20 or 40 min at 98°C in 1 mmol/L ethylenediamine tetraacetic acid and 10 mmol/L Tris–HCl buffer (pH 7.0 or 9.0; Table 1). Before staining, the sections were incubated in methanol containing 3% H_2O_2 for 10 min to block endogenous peroxidase activity. To reduce non-specific binding of the second-layer reagent, the preparations were pre-incubated with normal serum corresponding to the species in which the second antibody was produced. Details of the primary antibodies and retrieval are listed in Table 1. The preparations were incubated overnight at 4°C in a moist chamber. Then, they were incubated with Simple Stain MAX-PO (Nichirei, Tokyo, Japan) for 30 min. The reactions were visualized by applying 3,3′-diaminobenzidine-tetrachloride (DAB; Dojindo, Kumamoto, Japan), and the sections were counterstained with hematoxylin and mounted.

Double immunofluorescence labeling

Double labeling of CD8 and granulysin, CD8 and granzyme B, CD8 and perforin, and CD3 and Foxp3 was performed. The deparaffinized sections were subjected to antigen retrieval, as described above. Granulysin, granzyme B, perforin and Foxp3 were labeled using Alexa Fluor 488 (Molecular Probes, Eugene, OR, USA) to give a green signal, whereas CD3 and CD8 were labeled with Cy3, which produces a red signal. When the species used to make both primary antibodies was the mouse, heat treatment was used.[9] The first step included sequential incubations with mouse monoclonal antibody against the first antigen at 37°C for 1 h and biotin-labeled antimouse rabbit serum at 37°C for 1 h, followed by the application of Alexa Fluor 488-labeled streptavidin at 37°C for 1 h. After the first step, the specimens were heated in 10 mmol/L citrate buffer (pH 6.0) at exactly 90°C for 15 min. Fluorescent dyes such as Alexa Fluor 488 and Cy3 are resistant to heating at 90°C for 15 min, and the antigenicity of the primary antibody was lost entirely.[9] For the second step, the specimens were incubated with mouse monoclonal antibody against the second antigen at 37°C for 1 h, and then Cy3-labeled antimouse goat serum was added at 37°C for 1 h. We have preliminarily confirmed that heat treatment with 10 mmol/L citrate buffer (pH 6.0) at 90°C for 15 min did not influence immunoreactivity in the second staining step. When the two signals were merged using DP Manager imaging software, double-positive areas were visualized as yellow.

Quantitative and statistical analyses

The number of cells positive for CD1a, CD3, CD4, CD8, CD68, granulysin, granzyme B, perforin or Foxp3 per five high-power fields (750 μm^2) in each case was counted using printed photographs. The ratio of granulysin$^+$ cells/CD8$^+$ cells, granzyme B$^+$ cells/CD8$^+$ cells, and perforin$^+$ cells/CD8$^+$ cells in each case was calculated. The data were expressed as the mean ± standard deviation (SD). Student's *t*-test (two-tailed) was used to evaluate the significance of differences between groups with equal SD, and Mann–Whitney *U*-tests (two-tailed) were applied to the groups with unequal SD.

RESULTS

Immunohistochemistry

Cytotoxic molecules such as granulysin, granzyme B and perforin were labeled within a large proportion of the inflammatory infiltrates in the epidermis, subepidermal blister and upper dermis of the SJS/TEN cases, whereas they were distributed sparsely in the interface and upper dermis of the EMM cases (Fig. 1). Inflammatory infiltrates expressing Foxp3 or CD4 in the epidermis and dermis

Table 1. Panel of primary antibodies and the methods of retrieval

				Retrieval			
Antibody	Species	Clone	Source	Method	pH	Condition	Dilution
Granulysin	Mouse	RJT48	Kamiya Biomedical	Microwave	9	98°C 40 min	1:100
Granzyme B	Mouse	11F1	Novocastra	Microwave	9	98°C 40 min	1:50
Perforin	Mouse	5B10	Novocastra	Microwave	9	98°C 40 min	1:25
Fox P3	Rabbit	(poly)	Spring Bioscience	Microwave	9	98°C 40 min	1:100
CD1a	Mouse	O10	DAKO	Microwave	9	98°C 40 min	1:100
CD3	Mouse	PS1	Novocastra	Microwave	9	98°C 20 min	1:100
CD4	Mouse	1F6	Novocastra	Microwave	9	98°C 20 min	1:80
CD8	Mouse	1A5	Novocastra	Microwave	7	98°C 20 min	1:100
CD68	Mouse	KP-1	DAKO	Proteinase-K	–	Room temperature 5 min	1:100