represents the average cytotoxicity for each patient. All the experiments were performed at least three times.

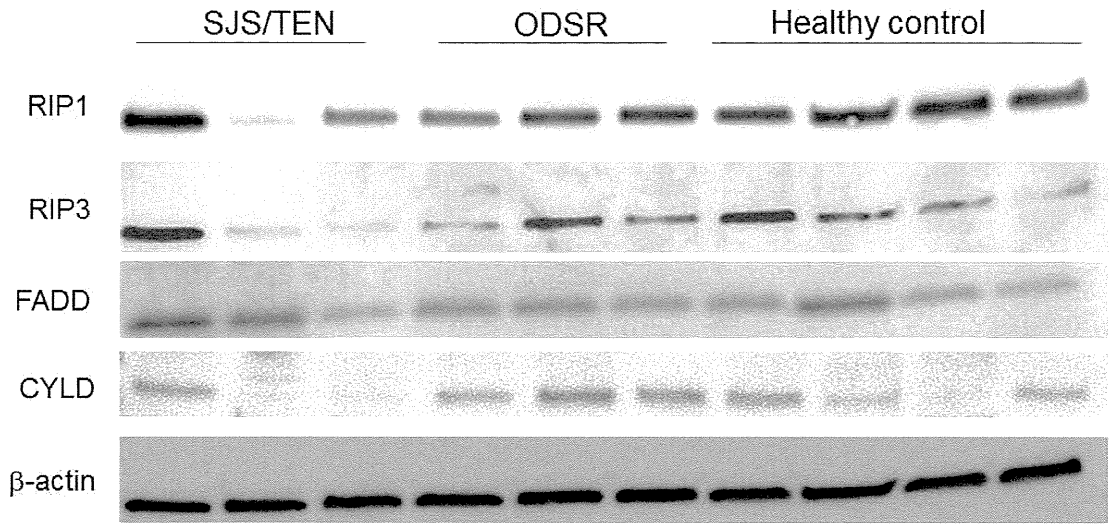

Fig. S5. Protein levels of necroptosis signaling molecules in keratinocytes from SJS/TEN patients, ODSR patients, or healthy controls. The expression of RIP1, RIP3, FADD and CYLD in keratinocytes was analyzed by immunoblotting. Keratinocytes were obtained from patients No. 3, 4, 5, 18, 19 and 20 and healthy controls No. 3, 4, 5 and 7.

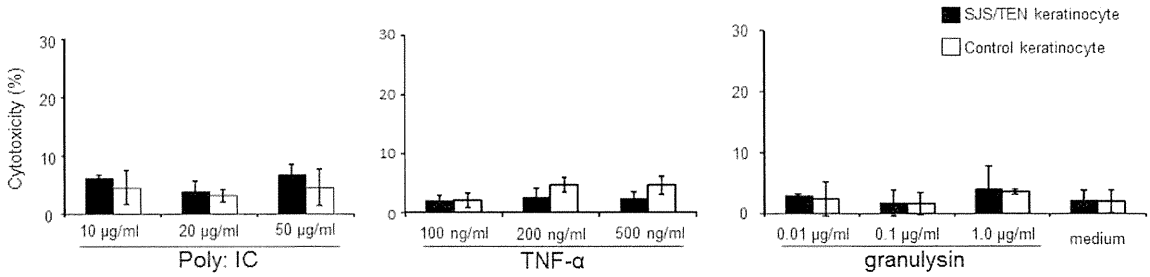

Fig. S6. Effect of poly(I:C), TNF-α, and granulysin on SJS/TEN keratinocyte cytotoxicity. Poly: IC (10, 20, 50 μg/ml), TNF-α (100, 200, 500 ng/ml) and granulysin (15-kD form) (0.01, 0.1, 1.0 μg/ml). Keratinocytes were obtained from patient No. 3 (post-lesional skin) and healthy control No. 5.

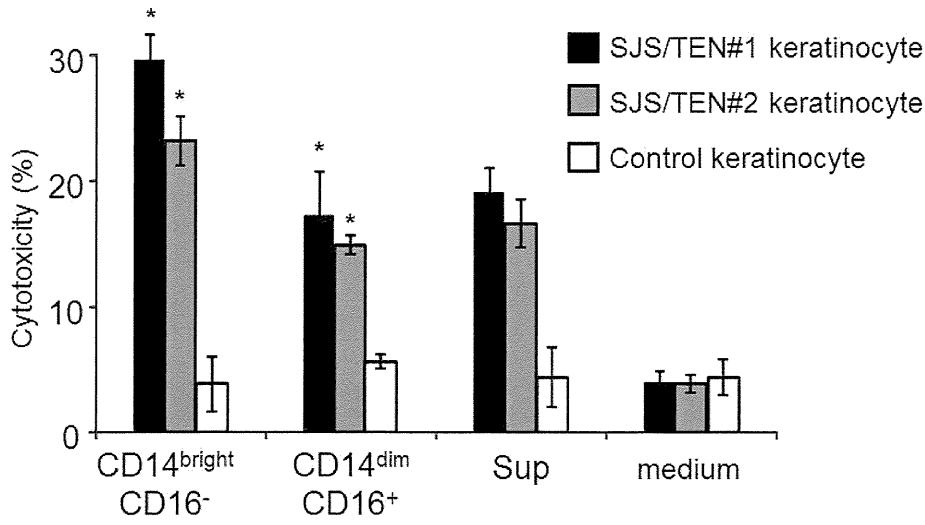

Fig S7. The cytotoxicity of CD14^{bright} CD16^{-} and CD14^{dim} CD16^{+} cells on SJS/TEN keratinocytes. Purified CD14^{bright} CD16^{-} and CD14^{dim} CD16^{+} cells were exposed to supernatant from CD14-depleted PBMCs and were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine and 25 mM HEPES buffer for 1 day. The supernatant was collected, and cytotoxicity was assessed. *$P < 0.05$ vs. medium. SJS/TEN#1 and #2 keratinocytes were obtained from patients No. 3 (post-lesional skin) and No. 5 (non-lesional skin), respectively. Healthy control No.3. PBMCs were obtained from patient No. 3.

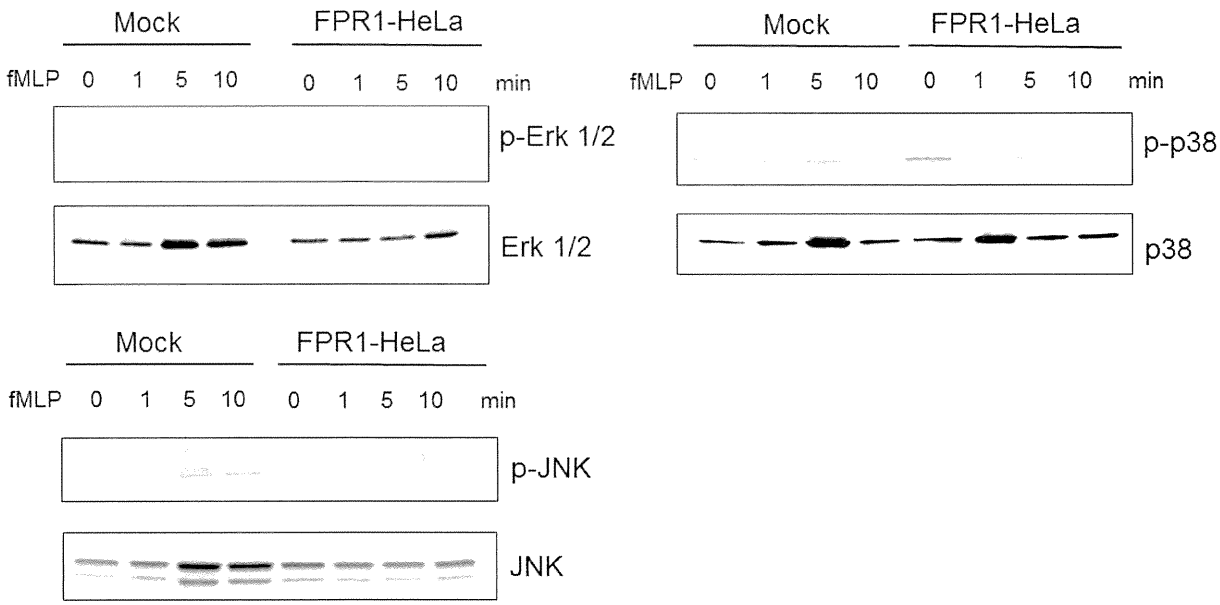

Fig. S8. FPR1 stimulation does not induce phosphorylation of JNK, p38, or Erk. Erk 1/2, p-38 and JNK phosphorylation were analyzed in FPR1-HeLa and mock-transfected cells treated with fMLP (5 nM). Cell lysates were analyzed by immunoblotting with anti-pErk 1/2, p-p38 and p-JNK antibodies.

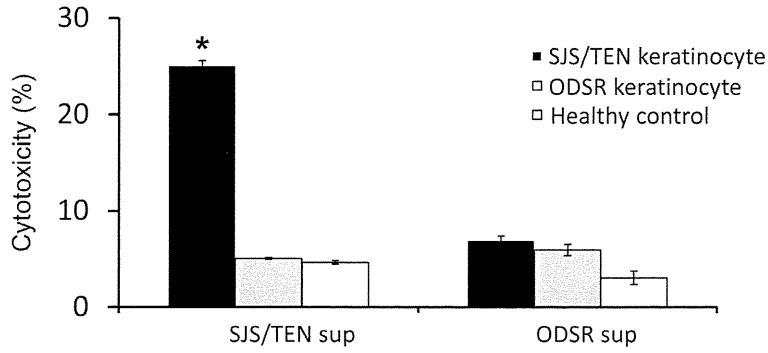

Fig. S9. Cytotoxicity in SJS/TEN keratinocytes, ODSR keratinocytes, or healthy control keratinocytes induced by PBMC supernatant, as measured by LDH assay. *P < 0.01 vs. ODSR and healthy control keratinocytes. Keratinocytes were obtained from patients No. 10 (post-lesional skin) and No. 19 (non-lesional skin) and healthy control No. 5. PBMCs were obtained from patients No. 3 and No. 14. *P < 0.01 vs. ODSR and healthy control keratinocytes.

Table S1. Mass spectrometry result of the proteins in SJS/TEN supernatant.

#	Identified Protein (126)	Accession #	SJS/TEN	ODSR#1	ODSR#2	medium
1	talin	gi|119578757 (+4)	53	0	0	0
2	myosin-9	gi|12667788 (+1)	43	0	0	0
3	filamin-A isoform 1	gi|116063573 (+5)	23	1	0	0
4	unnamed protein product	gi|21752646 (+2)	18	0	0	0
5	Chain A, Crystal Structure Of Human Enolase	gi|203282367 (+10)	13	2	3	0
6	fibrinogen alpha chain isoform alpha preproprotein	gi|11761629 (+5)	11	0	0	0
7	vinculin	gi|119574932 (+13)	10	0	0	0
8	78-kDa glucose-regulated protein precursor	gi|16507237 (+4)	9	0	0	0
9	unnamed protein product	gi|158256710 (+6)	8	0	0	0
10	Alpha-actinin-1	gi|122146006 (+27)	8	1	0	0
11	hemoglobin	gi|109893891 (+56)	8	0	2	0
12	glyceraldehyde-3-phosphate dehydrogenase	gi|31645 (+3)	8	0	0	0
13	moesin	gi|119625804 (+3)	7	0	0	0
14	Heat shock cognate 71-kDa protein	gi|123647 (+31)	7	0	0	0
15	tubulin beta-1 chain	gi|13562114	6	0	0	0
16	aldolase A, fructose-bisphosphate	gi|119600342 (+5)	6	0	0	0
17	calelectrin	gi|179976 (+8)	5	0	0	0
18	ATP synthase, H^+ transporting, mitochondrial F1	gi|127798841	5	0	0	0

	complex, alpha subunit 1, cardiac muscle	(+9)				
19	CAP, adenylate cyclase-associated protein 1	gi|119627645 (+6)	4	0	0	0
20	unnamed protein product	gi|193787540 (+14)	4	0	0	0
21	unnamed protein product	gi|189055002 (+5)	4	0	0	0
22	keratin 1	gi|11935049 (+2)	4	22	6	19
23	Chain A, Three-Dimensional Structure Of A Transglutaminase: Human Blood Coagulation Factor Xiii	gi|1127268 (+15)	3	0	0	0
24	annexin A1	gi|119582950 (+7)	3	0	0	0
25	Chain A, Human Annexin A2 With Heparin Tetrasaccharide Bound	gi|114794644 (+16)	3	0	0	0
26	Putative HLA class I histocompatibility antigen, alpha chain H	gi|308153595 (+1)	3	0	0	0
27	GDP dissociation inhibitor 2	gi|119606836 (+9)	3	0	0	0
28	Chain A, Crystal Structure Of Human Phosphoglycerate Kinase Bound To D-Adp	gi|193506632 (+12)	2	0	1	0
29	albumin	gi|11493459 (+19)	2	3	0	0
30	coronin-like protein	gi|1002923 (+6)	2	0	0	0
31	malate dehydrogenase 1, NAD (soluble)	gi|119620368 (+3)	2	0	0	0
32	ribonuclease/angiogenin inhibitor 1	gi|119622735 (+6)	2	0	0	0
33	Chain R, Twinning In Crystals Of Human Skeletal Muscle D- Glyceraldehyde-3-Phosphate Dehydrogenase	gi|230867	2	0	0	0
34	peptidyl-prolyl cis-trans isomerase A	gi|10863927	2	0	0	0

		(+53)				
35	hemoglobin beta	gi|229149	2	0	0	0
36	clathrin, heavy polypeptide (Hc)	gi|119614801 (+36)	2	0	0	0
37	Chain A, Glutathione Transferase P1-1	gi|11514451 (+23)	2	0	0	0
38	hCG2016942	gi|119594944 (+15)	2	0	0	0
39	Chain A, Structure Of The Aflatoxin Aldehyde Reductase In Complex With Nadph	gi|157881389 (+6)	2	0	0	0
40	ubiquitin-like modifier-activating enzyme	gi|23510338 (+4)	2	0	0	0
41	Chain A, Human Muscle L-Lactate Dehydrogenase M Chain	gi|13786849 (+6)	1	2	0	0
42	fibrinogen beta chain	gi|119625338 (+21)	1	0	0	0
43	acetyl-Coenzyme A acyltransferase 2	gi|119583356 (+6)	1	0	0	0
44	Chain A, Crystallographic And Kinetic Studies Of Human Mitochondrial Acetoacetyl-Coa Thiolase (T2)	gi|145579602 (+5)	1	0	0	0
45	Protein disulfide-isomerase	gi|110815912 (+42)	1	0	0	0
46	Parvin, beta	gi|127801538 (+5)	1	0	0	0
47	hypothetical protein DKFZp762H157.1	gi|11276938 (+31)	1	0	0	0
48	SAM domain and HD domain 1	gi|22209036 (+4)	1	0	0	0
49	septin 6	gi|119610263 (+19)	1	0	0	0
50	92 kDa type IV collagenase	gi|177205 (+7)	0	1	1	0
51	Chain A, Solution Structure Of Human Normal Adult Hemoglobin	gi|157883730 (+17)	0	0	1	0

52	Chain A, Structural Changes Of The Active Site Cleft And Different Saccharide Binding Modes In Lysozyme	gi|1065033 (+173)	0	0	1	0
53	epidermal cytokeratin 2	gi|181402 (+1)	0	0	2	1
54	Keratin 14	gi|12803709 (+7)	0	0	0	1
55	keratin, type I cytoskeletal 9	gi|55956899	0	10	3	8

Table S2. Patient and healthy control information.

Case	Age/sex	Causative drug	Type of cutaneous adverse reaction
1	56/F	Excemide	TEN
2	55/M	Acetaminophen	TEN
3	37/F	Amoxicillin	SJS
4	70/M	Ambroxol Hydrochloride	SJS
5	27/F	Lamotrigine	SJS
6	49/F	Acetaminophen	SJS
7	49/M	Benzbromarone	SJS
8	71/M	Phenytoin	SJS
9	68/M	Acetaminophen	SJS
10	79/F	Lansoprazole	SJS
11	50/F	Phenytoin	Maculopapular
12	30/M	Lamotrigine	Maculopapular
13	37/F	Amoxicillin	Maculopapular
14	64/M	Levothyroxine Sodium Hydrate	Maculopapular
15	65/M	Sulfamethoxazole, Trimethoprim	Maculopapular
16	70/M	Carbamazepine	Maculopapular
17	24/F	Carbamazepine	Maculopapular
18	75/F	Celecoxib	Maculopapular
19	63/F	Isoniazid sodium methanesulfonate hydrate	Maculopapular
20	61/F	Zonisamide	Maculopapular
21	8/M	Acetaminophen	Maculopapular
22	17/F	Lamotrigine	DRESS/DIHS
23	70/M	Carbamazepine	DRESS/DIHS
24	61/F	Sulfamethoxazole, Trimethoprim	DRESS/DIHS
25	65/M	Sulfamethoxazole, Trimethoprim	DRESS/DIHS
26	80/M	Carbamazepine	DRESS/DIHS

Case	Age/sex	
1	6/M	Healthy control
2	33/F	Healthy control
3	35/M	Healthy control
4	40/F	Healthy control
5	47/M	Healthy control
6	56/M	Healthy control
7	56/F	Healthy control
8	62/M	Healthy control
9	68/F	Healthy control
10	80/F	Healthy control

Data File S1. The promoter region of FPR1 has no pathogenic mutations.

Nucleotides from the –88 to the –72 position (–88/–72) of FPR1 were shown to influence its transcriptional activity (*31*), if the nucleotide number is designated as its position relative to the guanidine (+1) at the transcriptional start site. Therefore, we hypothesized that mutations within the promoter region of FPR1, especially the –88/v72 region, could increase FPR1 production and might be involved in the pathogenesis of SJS/TEN. To address this hypothesis Japanese patients with SJS/TEN and ODSR were recruited (8 patients and 7 patient, respectively), and genomic DNA was isolated from their peripheral blood. Participants gave written informed consent in compliance with the Declaration of Helsinki Principles. This study was approved by the Medical Ethics Committee of Hokkaido University, Sapporo, Japan. For mutation analysis of the promoter region of FPR1, nucleotides from the –630 to the +117 position (–630/+117) were amplified and sequenced as described previously (*31*), but no mutation except c.-533G>A (rs59390147) was identified in the –630/+117 region in any of the patients with either SJS or ODSR. The mutation c.-533G>A (rs59390147) was carried by two and five patients with SJS and ODSR, respectively. The lack of statistically significant difference between the two groups with respect to the SNP highly suggests that mutations in the promoter region of FPR1 are not associated with the pathogenesis of SJS, at least in the Japanese population.

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Original Article

Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions

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Aim: Telaprevir-based therapy for chronic hepatitis C patients is effective; however, the high prevalence of dermatological reactions is an outstanding issue. The mechanism and characteristics of such adverse reactions are unclear; moreover, predictive factors remain unknown. Granulysin was recently reported to be upregulated in the blisters of patients with Stevens–Johnson syndrome (SJS). Therefore, we investigated the risk factors for severe telaprevir-induced dermatological reactions as well as the association between serum granulysin levels and the severity of such reactions.

Methods: A total of 89 patients who received telaprevir-based therapy and had complete clinical information were analyzed. We analyzed the associations between dermatological reactions and clinical factors. Next, we investigated the time-dependent changes in serum granulysin levels in five and 14 patients with grade 3 and non-grade 3 dermatological reactions, respectively.

Results: Of the 89 patients, 57 patients had dermatological reactions, including nine patients with grade 3. Univariate analysis revealed that grade 3 dermatological reactions were significantly associated with male sex. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Three patients with grade 3 dermatological reaction had severe systemic manifestations including SJS, drug-induced hypersensitivity syndrome, and systemic lymphoid swelling and high-grade fever; all were hospitalized. Importantly, among the three patients, two patients' serum granulysin levels exceeded 8 ng/mL at onset and symptoms deteriorated within 6 days.

Conclusion: Male patients are at high risk for severe telaprevir-induced dermatological reactions. Moreover, serum granulysin levels are significantly associated with the severity of dermatological reactions and may be a predictive factor in patients treated with telaprevir-based therapy.

Key words: drug-induced hypersensitivity syndrome, granulysin, hepatitis C virus, telaprevir, toxic epidermal necrolysis

INTRODUCTION

HEPATITIS C IS a major pathogen causing liver cirrhosis and hepatocellular carcinoma worldwide. Until recently, standard therapies for chronic hepatitis C virus (HCV) genotype 1 infection were based on the combination of pegylated interferon (PEG IFN) and ribavirin (RBV); these combination therapies yield a sustained virological response (SVR) rate of approximately 50%.[1] Several classes of novel direct-acting antivirals

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(DAA) were recently developed and tested in clinical trials. Two first-generation HCV NS3/4A protease inhibitors, boceprevir[2,3] and telaprevir,[4–6] have been approved for the treatment of genotype 1 HCV infection. The inclusion of these agents in HCV treatment regimens has led to large improvements in treatment success rates.

Telaprevir, the first DAA, is administrated in combination with PEG IFN and RBV for 24 weeks, resulting in SVR rates up to 70–80%.[4,6–8] Although the telaprevir combination regimen is highly effective, the high frequency and severity of adverse events are outstanding issues limiting its use. Dermatological reactions are particularly prevalent, developing in 56–84.6% of patients treated with telaprevir, PEG IFN and RBV combination therapy.[9,10] Moreover, the prevalence of severe dermatological reactions including Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome (DIHS) are substantially higher in patients treated with telaprevir-based therapy than PEG IFN and RBV combination therapy.[8,10] McHutchison *et al.* reported that 7% of patients treated with telaprevir, PEG IFN and RBV combination therapy discontinue therapy because of rash or pruritus in contrast to only 1% of patients treated with PEG IFN and RBV.[8] In some patients, serious skin reactions persist even after stopping all drugs.[10] However, the pathogenesis and clinical predictors of these adverse reactions are poorly understood.

Granulysin is a 15-kDa cationic cytolytic protein released by cytotoxic T lymphocytes and natural killer cells that induces apoptosis in target cells and has antimicrobial activities.[11] Serum levels of granulysin are elevated in primary virus infections including Epstein–Barr virus and parvovirus B19.[12] It was recently reported that serum granulysin levels are significantly elevated in patients with several types of severe dermatological lesions including SJS/TEN, which is the characteristic serious adverse event in telaprevir-containing regimens.[13,14]

Accordingly, the present study determined the risk factors for severe dermatological reactions in patients receiving telaprevir, PEG IFN and RBV combination therapy as well as the association between serum levels of granulysin and severe dermatological reactions.

METHODS

Patients and methods

IN THIS RETROSPECTIVE case–control study, at Hokkaido University Hospital and associated hospitals in the NORTE Study Group, between December 2011 and November 2013, a total of 123 patients positive for HCV genotype 1 with high serum HCV RNA titer (>5 log IU/mL) received PEG IFN, RBV and telaprevir combination therapy. Patients were excluded if they required hemodialysis or had a positive test result for serum hepatitis B surface antigen, co-infection with other HCV genotypes or HIV, evidence of autoimmune hepatitis or alcoholic hepatitis, or malignancy. Serum granulysin levels were analyzed in five healthy volunteers with no HCV, HIV or hepatitis B virus infection or any inflammatory diseases.

Written informed consent according to the process approved by the hospital's ethics committee was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of each participating hospital.

Study design and treatment regimen

Telaprevir 500 or 750 mg was typically administrated every 8 h after meals for 12 weeks. PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) 1.5 IU/kg was administrated s.c. once per week for 24 weeks. RBV (Rebetol; MSD) was administrated for 24 weeks in two divided daily doses according to bodyweight: 600, 800 and 1000 mg for patients with bodyweights of less than 60, 60–80 and more than 80 kg, respectively. The doses of PEG IFN-α-2b, RBV and telaprevir were reduced at the attending physician's discretion on the basis of hemoglobin levels, decreased white blood cell or platelet counts, or adverse events.

During treatment, patients were assessed as outpatients at weeks 1, 2, 4, 6 and 8, and then every 4 weeks thereafter for the duration of treatment. Physical examinations and blood tests were performed at all time points.

Outcomes

The primary end-point was SVR, which was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. The secondary end-points were end-of-treatment virological responses (HCV RNA undetectable in serum) and rapid virological response (RVR), which was defined as undetectable serum HCV RNA at 4 weeks after the start of treatment. Dermatological reactions were classified according to severity in the same manner as in phase III trials in Japan.[10]

Serum granulysin measurement

To evaluate serum granulysin levels in chronic hepatitis C, we first measured serum granulysin levels in five

healthy volunteers and compared them with those of 20 chronic hepatitis C patients before treatment. Serum granulysin levels were measured at the onset of dermatological reactions (within 3 days of onset); if the symptoms worsened, the time when worsening occurred was adopted. Meanwhile, in patients with no dermatological reactions, the highest serum granulysin level during treatment was adopted.

Serum granulysin levels were measured by a sandwich enzyme-linked immunosorbent assay as described previously.[12,14,15] Briefly, plates coated with 5 mg/mL mouse antibody against human granulysin, RB1 antibody, were washed with phosphate-buffered saline containing 0.1% Tween-20. Next, they were blocked with 10% fetal bovine serum in washing buffer at room temperature for 2 h. The samples and standards (Recombinant Granulysin; R&D Systems, Minneapolis, MN, USA) were incubated for 2 h at room temperature. Next, they were reacted with 0.1 mg/mL biotinylated mouse antibody against human granulysin, RC8 antibody. The plates were subsequently treated with horseradish peroxidase-conjugated streptavidin (Roche Diagnostics, Basel, Switzerland). The plates were then incubated with tetramethyl-benzidine substrate (Sigma, St Louis, MO, USA), and 1 M sulfuric acid was then added. The optical density was measured at 450 nm using a microplate reader.

Diagnosis of dermatological reactions

Dermatological reactions were investigated throughout the 24-week administration period in the telaprevir-based combination therapy. Dermatological reactions were classified according to severity as follows. Grade 1 was defined as involvement of less than 50% of the body surface and no evidence of systemic symptoms. Grade 2 was defined as involvement of less than 50% of the body surface but with multiple or diffuse lesions or rashes with characteristic mild systemic symptoms or mucous membrane involvement with no ulceration/erosion. Grade 3 was defined as a generalized rash involving 50% or more of the body surface or a rash with any new significant systemic symptoms and considered to be related to the onset and/or progression of the rash. Life-threatening reactions included SJS, TEN, drug rash with eosinophilia and systemic symptoms (DRESS)/DIHS, erythema multiforme and other life-threatening symptoms, or patients presenting with features of serious disease.

When adverse skin reactions were detected, the attending physician classified the degree of severity and referred the patients to a dermatologist as needed. In principal, when grade 3 dermatological reactions occurred, the attending physician referred the patient to a dermatologist and discontinued telaprevir. When severe dermatological reactions including SJS/TEN and DRESS/DIHS were suspected, all drugs were discontinued immediately. SJS/TEN and DIHS were diagnosed by skin biopsy and according to disease criteria, respectively.

Statistical analysis

Categorical and continuous variables were analyzed by the χ^2-test and the unpaired Mann–Whitney *U*-test, respectively. All *P*-values were two-tailed, and the level of significance was set at $P < 0.05$. Multivariate logistic regression analysis with stepwise forward selection included variables showing $P < 0.05$ in univariate analyses.

The association between dermatological reactions and serum granulysin levels were evaluated by one-way ANOVA followed by Tukey's honestly significant difference test. All statistical analyses were performed using SPSS version 21.0 (IBM Japan, Tokyo, Japan).

RESULTS

Patients

WE INCLUDED 123 chronic hepatitis C patients who received telaprevir-based triple therapy. Of these, 89 patients who had proper information of dermatological adverse events were included. The baseline characteristics of patients are shown in Table 1.

Of these 89 patients, time-dependent changes of serum granulysin concentrations were measured in 20 who had had conserved serum, at least, at the pretreatment point, 1 and 2 weeks after commencement of therapy, 1 and 2 months after commencement of therapy, the onset point of dermatological adverse reaction and the worsening point if symptoms became worse.

Among the 89 patients, 64% (57/89) developed dermatological reactions, including nine with grade 3 reactions (Table 2). The characteristics of dermatological reactions by grade are shown in Table 2. Non-grade 3 dermatological reactions tended to occur early during treatment compared to grade 3 dermatological reactions.

Association between dermatological reactions and treatment outcomes

First, we determined whether dermatological reactions were associated with final treatment outcomes.

Table 1 Baseline characteristics of the participating patients

Total number	89
HCV genotype 1b (1b/others)	89/0
Age (years)†	60.0 (19–73)
Sex (male/female)	48/41
Bodyweight (kg)†	63.0 (32–97)
Baseline white blood cell count (/μL)†	4800 (1500–9800)
Baseline hemoglobin level (g/dL)†	13.5 (9.9–16.7)
Baseline platelet count ($\times 10^3$)†	15.9 (6.6–86)
Baseline ALT level (IU/L)†	40 (15–300)
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.5 (3.2–7.6)
Initial telaprevir dose (1500/2250 mg)	20/89
Initial PEG IFN dose (1.5/<1.5 μg/kg)	775/14
Initial RBV dose (mg/kg)†	9.8 (2.2–15.5)
IL28B gene (rs8099917) (TT/non-TT/ ND)	51/22/16
HCV 70 core mutation (wild/ mutant/ND)	43/24/22
Previous treatment (naïve/relapse/NVR)	40/38/11

†Data are shown as median (range) values.
ALT, alanine transaminase; HCV, hepatitis C virus; IL28B, interleukin 28B; ND, not done; PEG IFN, pegylated interferon; RBV, ribavirin.

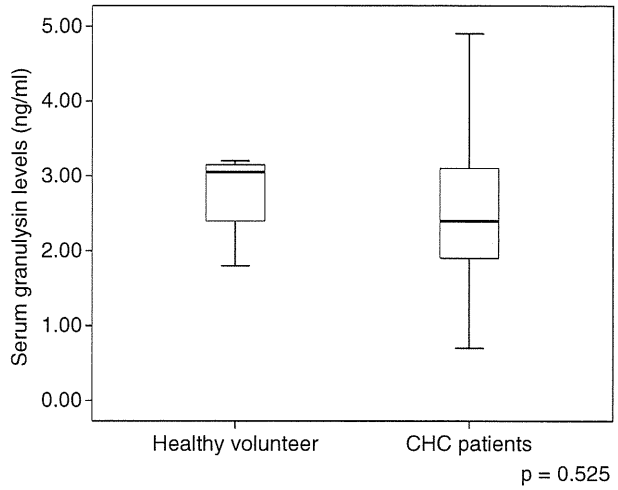

Figure 1 Serum granulysin levels of healthy volunteers and chronic hepatitis C patients. Serum granulysin levels were compared between five healthy volunteers and untreated 20 chronic hepatitis C patients. $P < 0.05$, Mann–Whitney *U*-test.

Univariate analyses identified baseline white blood cell and platelet counts, RVR, and non-grade 3 dermatological reactions significantly associated with SVR (Table 3). Among the nine patients with grade 3 dermatological reactions, three discontinued all treatment and six discontinued telaprevir administration; SVR was achieved in zero of the three (0%) and two of the six (33%), respectively.

Multivariate analysis showed that RVR and non-grade 3 dermatological reactions were significantly associated with SVR (Table 3).

Analysis of risk factors for telaprevir-induced dermatological reactions

Next, we analyzed the association between severe (i.e. grade 3) dermatological reactions and clinical parameters (Table 4). Univariate analysis showed that only sex was significantly associated with the grade 3 dermatological reactions ($P = 0.03$).

Serum granulysin levels in healthy subjects and chronic hepatitis C patients

As shown in Figure 1, serum granulysin levels did not differ significantly between healthy volunteers and chronic hepatitis C patients. Next, we evaluated the association between the severity of dermatological reactions and serum peak granulysin levels in 20 patients including five, four, five and six with grades 1, 2 and 3, and no dermatological events, respectively. One-way ANOVA showed that serum granulysin level was significantly associated with the severity of dermatological reactions ($P = 0.036$); in addition, Tukey's honestly significant difference test revealed that the serum

Table 2 Characteristics of the patients with each dermatological adverse event grade

	n	Age†	Sex (male/female)	Initial telaprevir dose (2250/1500)	Onset of DAR (days)
No DAR	32	61 (28–72)	15/17	26/6	
Grade 1	32	58 (19–73)	15/17	24/8	7 (3–50)
Grade 2	16	61 (44–73)	10/6	12/4	3.5 (1–56)
Grade 3	9	61 (48–65)	8/1	8/1	22 (1–60)

†Data are shown as median range) values.
DAR, dermatological adverse reaction

Table 3 Comparison of the clinical and laboratory characteristics of the patients with HCV infection based on therapeutic response

All patients	SVR	Non-SVR	Univariate analysis	Multivariate analysis		
$n = 89$	$n = 68$	$n = 21$	P	OR	95% CI	P
Age (years)†	60 (19–73)	62 (28–73)	0.402			
Sex (male/female)	37/31	11/10	0.870			
Bodyweight (kg)†	62 (39–97)	64 (32–87)	0.761			
Baseline white blood cells (/μL)†	5135 (1500–9800)	4200 (2490–7200)	0.048	0.492	(0.121–1.993)	0.320
Baseline hemoglobin level (g/dL)†	13.5 (10.5–16.7)	12.1 (9.9–15.4)	0.862			
Baseline platelet count ($\times 10^3$)†	16.7 (6.6–31.5)	12.8 (7.2–86)	0.025	0.388	(0.093–1.614)	0.193
Baseline ALT level (IU/L)†	37 (15–300)	53 (23–159)	0.070			
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.7 (3.2–7.6)	6.4 (5.7–7.3)	0.812			
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.7 (0.5–0.9)	0.433			
Initial telaprevir dose (1500/2250 mg)	52/16	17/4	0.460			
Initial PEG IFN dose (1.5/<1.5 μg/kg)	58/10	17/4	0.430			
Initial RBV dose (mg/kg)†	9.9 (2.2–15.5)	9.5 (4.4–12.5)	0.546			
IL28B gene (rs8099917) (TT/non-TT/ND)	43/15/10	8/7/6	0.107			
Core 70 a.a. mutation (wild/mutant/ND)	36/16/16	7/8/6	0.108			
Previous treatment (naive/relapse/NVR)	34/28/6	6/10/5	0.095			
Rapid virological response (+/−)	60/8	10/11	<0.001	10.89	(2.838–41.83)	0.001
Grade 3 DAR (−/+)	66/2	14/7	<0.001	27.44	(3.718–202.5)	0.001

†Data are shown as median (range) values.
a.a., amino acid; ALT, alanine transaminase; CI, confidence interval; Cr, creatinine; DAR, dermatological adverse reaction; HCV, hepatitis C virus; IL28B, interleukin 28B; ND, not done; NVR, non-virological response; OR, odds ratio; PEG IFN, pegylated interferon; SVR, sustained virological response; RBV, ribavirin.

granulysin levels of patients with grade 3 dermatological reactions were significantly higher than those of patients with grade 1 or no dermatological reactions (both $P < 0.05$, Fig. 2).

Time-dependent changes in serum granulysin levels

We investigated the time-dependent changes in serum granulysin levels in five and 15 patients with grade 3 and non-grade 3 dermatological reactions, respectively (Fig. 3). Serum granulysin levels of patients with non-grade 3 dermatological reactions never exceeded 10 ng/ml. Of the five patients with grade 3 reactions, three had severe systemic manifestations that necessitated hospital admission: one each had SJS, DIHS, and systemic lymphoid swelling and high fever (>39 °C). All patients with grade 3 dermatological reactions with systemic manifestations had peak serum granulysin levels exceeding 10 ng/mL; importantly, the serum granulysin levels of two patients already exceeding 8 ng/mL at the onset of the reactions worsened within 6 days.

DISCUSSION

THE PRESENT STUDY demonstrates a significant association between telaprevir-induced dermatological reactions and elevated serum granulysin levels for the first time. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Thus, the results indicate that serum granulysin level seems to be a useful predictor of telaprevir-induced dermatological reactions. Because the emergence of grade 3 dermatological reactions was significantly associated with non-SVR (Table 3), probably associated with high rate of treatment discontinuation, it is important to predict dermatological events in the early stage to achieve good treatment outcomes.

Table 4 Comparison of the clinical and laboratory characteristics of the patients based on the presence or absence of at least a grade 3 dermatological adverse event

All patients $n = 89$	Non-grade 3 $n = 80$	Grade ≥3 $n = 9$	Univariate analysis P
Age (years)†	60 (19–73)	61 (48–65)	0.453
Sex (male/female)	40/40	8/1	0.027
Bodyweight (kg)†	62 (32–97)	64 (51–87)	0.593
Baseline white blood cell count (/μL)†	4900 (1500–9800)	4700 (3000–7000)	0.876
Baseline hemoglobin level (g/dL)†	13.5 (9.9–16.7)	14.4 (12.1–15.4)	0.196
Baseline platelet count ($\times 10^3$)†	16.0 (6.6–86.0)	13.5 (10.4–22.5)	0.605
Baseline ALT level (IU/L)†	40(15–300)	37 (23–87)	0.765
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.8 (0.6–0.9)	0.123
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.6 (3.2–7.6)	6.4 (5.7–7.1)	0.465
Initial telaprevir dose (1500/2250 mg)	62/18	7/2	0.675
Initial telaprevir/bodyweight (mg/kg)	33.7 (20–71.4)	30.0 (23.6–44.1)	0.563
Initial PEG IFN dose (1.5/<1.5 μg/kg)	66/14	9/0	0.198
Initial RBV dose (mg/kg)†	9.7 (2.2–15.5)	10.7 (7.7–12.9)	0.161
IL28B gene (rs8099917) (TT/non-TT/ND)	47/19/14	4/3/2	0.353
Core 70 a.a. mutation (wild/mutant/ND)	38/22/20	5/2/2	0.511
Previous treatment (naïve/relapse/NVR)	35/36/9	5/2/2	0.972
Onset of dermatological AE (days)	5 (1–75)	22 (1–60)	0.352

†Data are shown as median (range) values.
a.a., amino acid; AE, adverse event; ALT, alanine transaminase; Cr, creatinine; HCV, hepatitis C virus; IL28B, interleukin 28B; NVR, non-virological response; PEG IFN, pegylated interferon; RBV, ribavirin.

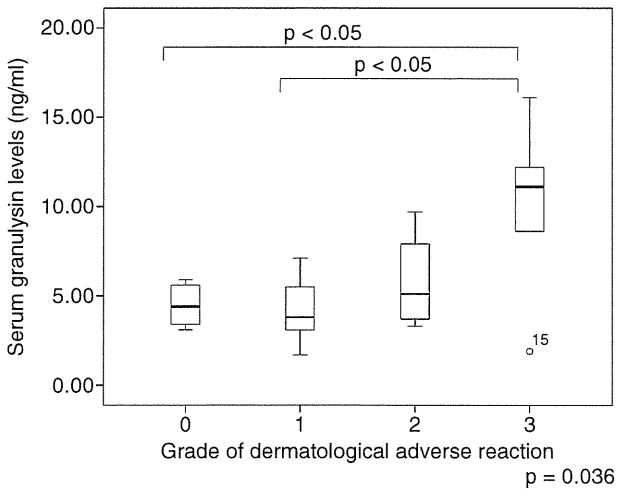

Figure 2 Association between dermatological adverse reaction severity and serum granulysin level. Serum granulysin levels were measured at the onset of dermatological reactions (i.e. within 3 days of onset); if the symptoms worsened, the time of worsening was adopted. In patients with no dermatological events, the highest serum granulysin level during treatment was adopted. $P < 0.05$, one-way ANOVA.

Recent genome-wide association studies have identified that genetic polymorphisms around the IL28B gene locus significantly associated with the outcome of PEG IFN and RBV combination therapy in HCV patients. Thus, PEG IFN and RBV combination therapy is ineffective in a subset of HCV-infected patients who have IL28B TG or GG genotypes, limiting the use of this therapy.[16] Therefore, novel drugs with different antiviral mechanisms were required. Accordingly, DAA were developed; they are mainly classified as NS3/4A protease inhibitors, or NS5B or NS5A inhibitors.[17] The NS3/4A serine protease inhibitor telaprevir, in combination with PEG IFN and RBV, has demonstrated the most promising results.[6–8] However, adverse events, especially severe dermatological reactions, develop more frequently in patients treated with telaprevir than those treated with only PEG IFN and RBV.

Little is known about the mechanisms of telaprevir-induced dermatological reactions. Reactions develop in patients treated with PEG IFN and RBV combination therapy[18,19] as well as telaprevir monotherapy.[20,21] It should be noted that the dermatological reactions in telaprevir monotherapy or PEG IFN and RBV therapy alone are generally mild.[7,8,20] However, dermatological

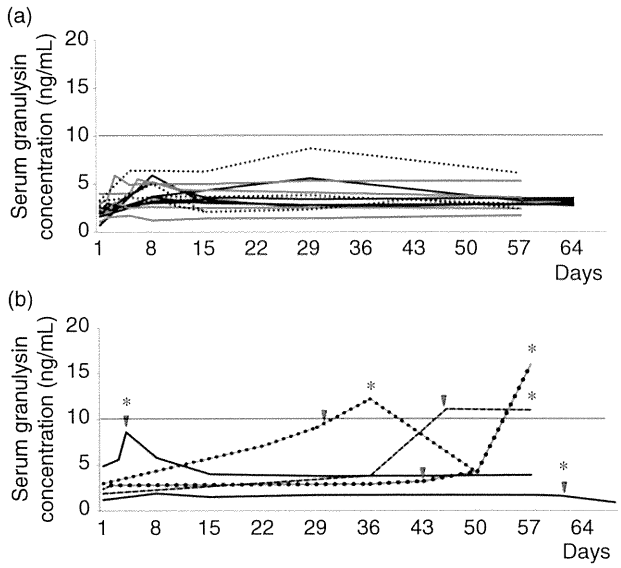

Figure 3 Association between time-dependent changes in serum granulysin levels and severe telaprevir-induced dermatological adverse reactions. (a) Time-dependent changes in serum granulysin levels patients with non-grade 3 dermatological reactions (three, five and six with grade 2, grade 1 and no reactions, respectively). The dashed line, gray line and black line indicate grade 2, grade 1 and no reaction, respectively. (b) Time-dependent changes in serum granulysin levels of five patients with grade 3 dermatological events. The dashed line indicates patients with severe systemic manifestations. Arrowheads indicate the onset of dermatological events and asterisks indicate the onset of grade 3 dermatological events.

reactions in telaprevir and PEG IFN/RBV combination therapy may be severe, indicating a synergistic effect. Severe dermatological events including SJS/TEN and DIHS have been reported in telaprevir-based triple therapy; these are life-threatening, and fatal cases have been reported.

The onset of grade 3 dermatological reactions tended to be later than non-grade 3 reactions, the same as in the study of Torii *et al.*[10] Taken together with the finding that male sex is a clinical risk factor, the results indicate that late-onset dermatological reactions in male patients treated with telaprevir-based triple therapy require more attention.

Roujeau *et al.* analyzed the risk factors for telaprevir-induced eczematous dermatitis and report that the incidence of telaprevir-related dermatitis was significantly higher age of more than 45 years, body mass index of less than 30 (kg/m^2), Caucasian ethnicity and treatment-naïve status.[9] While they analyzed the risk factors for telaprevir-induced eczematous dermatitis, the present study focused on the risk factors for severe telaprevir-induced dermatological reactions, because such reactions can affect treatment outcome (Table 2) and can be fatal. As mentioned above, male sex was significantly associated with grade 3 dermatological reactions. Sex is reported to be associated with the prevalence of some kinds of severe drug-induced dermatological events, although the underlying mechanism remains unknown.[22]

Fujita *et al.* report that serum granulysin levels are significantly elevated in SJS/TEN patients and thus may be a good predictive factor.[14] Therefore, we hypothesized that in telaprevir-based triple therapy for chronic hepatitis C patients, serum granulysin levels are associated with the severity of dermatological reactions and may thus be a predictive biomarker. However, Ogawa *et al.* report that serum granulysin levels also increase as a result of primary virus infections such as Epstein–Barr virus or parvovirus B19.[12] Thus, it remains unclear whether and how chronic viral infections, especially HCV, affect serum granulysin levels. In the present study, we compared serum granulysin levels between healthy volunteers and chronic hepatitis C patients; the results show that chronic HCV infection was not associated with serum granulysin levels (Fig. 1).

Chung *et al.* have reported that granulysin is the most highly expressed cytotoxic molecule in blisters of SJS/TEN and that massive keratinocyte death was induced by granulysin.[11] Fujita *et al.* reported that serum granulysin levels increased in the early stage of SJS/TEN caused by drugs including carbamazepine, imatinib and phenytoin.[14] Taken together with our results, we speculate that granulysin may be involved in the pathogenesis of early stage telaprevir-mediated dermatological adverse reactions possibly through induction of keratinocyte death.

Of five patients with grade 3 reactions, two patients without severe systemic manifestations did not have elevated serum granulysin of more than 10 ng/mL or did not have elevated levels before symptoms worsened. On the contrary, three patients with severe systemic manifestations had peak serum granulysin levels exceeding 10 ng/mL, and the symptoms of two patients with serum granulysin levels already exceeding 8 ng/mL at onset and within 6 days worsened. Therefore, serum granulysin tests may predict grade 3 dermatological adverse reaction with systemic manifestations. Furthermore, if serum granulysin levels elevate more than 8 ng/mL, more attention should be paid.

In Western countries, the prevalence of dermatological reactions in patients treated with telaprevir-based and

PEG IFN/RBV therapy are reported to be approximately 55% and 33%, respectively;[9,23] meanwhile, in Japanese patients, the respective rates are 74.9% and 58.7%. Moreover, approximately 4% and 9% of patients in Western and Japanese patients develop grade 3 reactions, respectively;[10] this is almost the same as that in the present study (10%). The difference may be due to genetic or ethnic variation. Therefore, genome-wide association studies may have identified a gene locus associated with telaprevir-induced severe dermatological reactions.

A limitation of this study is that the number of patients with grade 3 dermatological reactions is relatively small. However, the serum granulysin levels of patients with grade 3 dermatological reactions were significantly higher than those of other patients. Also, in two of the three patients with severe dermatological reactions, the serum granulysin level elevated before symptoms worsened, which are novel findings. Further study is required.

Triple therapy with the second-generation protease inhibitor simeprevir is reported to result in a similar prevalence of adverse reactions as PEG IFN and RBV combination therapy.[24,25] However, simeprevir is not approved worldwide. Although simeprevir-based triple therapy is effective, only 36–53% of prior non-responders achieve SVR.[24] Shimada *et al.* recently reported that by extending PEG IFN and RBV therapy from 24 to 48 weeks, telaprevir-based triple therapy improves the SVR to up to 68% in prior null responders.[26] Thus, telaprevir is a therapeutic option for prior null responders.

In conclusion, the present study suggests that male sex is a significant risk factor for severe telaprevir-induced dermatological reactions. In addition, serum granulysin levels are significantly associated with the severity of dermatological reactions and thus may be a good predictor of severe dermatological reactions with systemic manifestations in patients treated with telaprevir-based triple therapy.

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