Table 2 continued.

Genotype	Amino Acid	Patients With KTD (n = 19)	Patients With Normal Tubular Function (n = 171)	*P* Value[a]
2759T → C, rs2125739	Ile920Thr			
T/T		15 (71.4)	131 (77.5)	
T/C		6 (28.6)	31 (18.3)	.488
C/C		0 (0)	7 (4.1)	
SLC22A6 (OAT1)				
180C → T, rs11568630				
C/C		18 (94.7)	164 (95.9)	
C/T		1 (5.3)	7 (4.1)	.577
T/T		0 (0)	0 (0)	
ABCB1 (P-glycoprotein)				
2677T → A/G, rs2032582	A:Ser893Thr G:Ser893Ala			
T/T		0 (0)	47 (27.5)	
T/A		3 (15.8)	14 (8.2)	
G/G		4 (21.1)	36 (21.1)	.002
G/T		8 (42.1)	46 (26.9)	
G/A		1 (5.3)	24 (14)	
A/A		3 (15.8)	4 (2.3)	

Abbreviation: KTD, kidney tubular dysfunction.

[a] By Fisher exact test.

Table 2 summarizes the distribution of genotypes at the *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A11*, and *ABCB1* genes in the 2 groups. All polymorphisms were in Hardy-Weinberg equilibrium with a cutoff *P* value of .001. In single SNP analysis, a higher percentage of patients with KTD were found among genotype CC at position −24 and genotype AA at position 1249 of *ABCC2*, compared to patients with other genotypes (−24 CC; 14.3% [in 18 of 126 patients] vs 1.6% [in 1 of 64 patients]; $P = .004$; 1249 AA; 42.9% [in 3 of 7 patients] vs 8.7% [in 16 of 183 patients]; $P = .023$), respectively. The percentage of patients with KTD was also higher among genotype AA at position 2677 of *ABCB1*, compared to patients with other genotypes (2677 AA; 42.9% [in 3 of 7 patients] vs. 8.7% [in 16 of 183 patients]; $P = .023$). KTD was marginally associated with genotype AA at position 559 and genotype GG at position 4976 of *ABCC4* ($P = .112$, and .090, respectively).

Association of Genotypes with KTD

Univariate analysis showed a significant association between KTD and patients with genotype CC at position −24 (OR, = 10.50; 95% CI, 1.369–80.55; $P = .024$) and patients with genotype AA at position 1249 (OR, 7.828; 95% CI, 1.609–38.10; $P = .011$) of *ABCC2* (Table 3). The risk for KTD was higher in patients with both genotype CC at position −24 and genotype AA at position 1249 (OR, 31.88; 95% CI, 3.131–324.5; $P = .003$). Genotype AA at position 2677 of *ABCB1* was also significantly associated with KTD (OR, 7.828; 95% CI, 1.609–38.10; $P = .011$). Furthermore, old age (per 1 year, OR, 1.165; 95% CI, 1.100–1.233; $P < .001$), low body weight (per 1 kg decrement, OR, 1.076; 95% CI, 1.021–1.135; $P = .007$), and low eGFR (per 1 mL/minutes/1.73 m^2 decrement, OR, 1.052; 95% CI, 1.016–1.090; $P = .004$) were also associated with KTD.

Multivariate analysis identified genotype CC at position −24 and genotype AA at position 1249 of *ABCC2* as independent risks for KTD after adjustment for sex, age, weight, eGFR, and hypertension (adjusted OR, = 20.08; 95% CI, 1.711–235.7; $P = .017$; adjusted OR, 16.21; 95% CI, 1.630–161.1; $P = .017$), respectively (Table 4). Patients with both of the abovementioned two homozygotes showed higher adjusted OR in multivariate analysis (adjusted OR, 38.44; 95% CI, 2.051–720.4; $P = .015$; Table 4). On the other hand, genotype AA at position 2677 of *ABCB1* was not significantly associated with KTD in multivariate analysis adjusted for the abovementioned variables (adjusted OR, 1.686; 95%CI, .163–17.43; $P = .661$).

Association of Haplotypes at ABCC2 and ABCC4 with KTD

Haplotype construction was performed with the 4 identified SNPs with $P < .10$ in univariate analysis: *ABCC2*, −24 C → T, 1249 G → A; *ABCC4*, 559 G → T, 4976T → C (Table 4). Haplotypes with frequency of >1% were analyzed. *ABCC2* haplotype CA was significantly associated with TDF-induced KTD (OR, 2.910; 95% CI, 1.295–6.221; $P = .011$), whereas *ABCC2* haplotype TG was a protective haplotype (OR, 0.098; 95% CI, .002–.603; $P = .003$). *ABCC4* haplotype TT was marginally

Table 3. Univariate Analysis of Risks for Kidney Tubular Dysfunction in Patients With HIV Infection Treated With Tenofovir

Characteristic	OR	95% CI	*P* Value
Female sex	1.844	.204–16.67	.586
Age per 1 year	1.165	1.100–1.233	<.001
Weight per 1 kg decrement	1.076	1.021–1.135	.007
CD4 count per 1/μL decrement	1.002	.999–1.004	.261
Baseline eGFR per 1 mL/minutes/1.73 m^2 decrement	1.052	1.016–1.090	.004
Concurrent use of nephrotoxic drugs	1.559	.322–7.555	.581
Hepatitis B	0.721	.156–3.319	.674
C-reactive protein per 1 mg/dL	1.551	.689–3.494	.289
Hypertension	2.234	.843–5.922	.106
Dyslipidemia	0.578	.183–1.823	.349
Duration of treatment with tenofovir disoproxil fumarate (weeks)	0.999	.992–1.007	.888
ABCC2			
–24 CC	10.50	1.369–80.55	.024
1249 AA	7.828	1.609–38.10	.011
–24 CC plus 1249 AA	31.88	3.131–324.5	.003
2934 GG	1.358	.167–11.07	.775
ABCC4			
559 TT	4.912	.837–28.81	.078
912 TT	1.466	.531–4.042	.460
2269 AA	2.756	.530–14.34	.228
3348 GG	1.950	.510–7.463	.329
4135 GG	1.254	.450–3.494	.665
4976 CC	2.462	.925–6.547	.071
ABCC10			
526 GG	1.158	.360–3.725	.805
2759 TT	0.619	.220–1.738	.363
ABCB1			
2677 AA	7.828	1.609–38.10	.011

Abbreviations: CI, confidence interval; eGFR: estimated glomerular filtration rate; HIV, human immunodeficiency virus; OR, odds ratio.

[a] Due to low prevalence of minor alleles, rs56220353, rs11568630, and rs2274407 were not included in this analysis.

associated with tenofovir-induced KTD (OR, 2.497; 95% CI, .902–6.949; $P = .077$).

DISCUSSION

The present study demonstrated that genotype CC at position −24 and genotype AA at position 1249 of *ABCC2* gene are associated with tenofovir-induced KTD in Japanese patients with HIV-1 infection. The effect of SNPs was more evident in patients with both −24 CC and 1249 AA homozygotes than in those with either homozygote only. The findings of this study resolve long-term controversy over the role of genetic

Table 4. Multivariate Analysis for the Risk of Tenofovir-Induced Kidney Tubular Dysfunction With Homozygotes at −24 and 1249 of *ABCC2* in Patients With HIV Infection

ABCC2	Adjusted OR	95% CI	*P* Value
Homozygote at −24 CC	20.08	1.711–235.7	.017
Homozygote at 1249 AA	16.21	1.630–161.1	.017
Homozygotes at −24 CC plus 1249 AA	38.44	2.051–720.4	.015

Each variable was adjusted for sex, age, weight, estimated glomerular filtration rate, and hypertension.

Abbreviations: CI, confidence interval; OR, odds ratio.

polymorphisms in tenofovir-induced KTD and confirm the effect of the SNPs in *ABCC2* gene in tenofovir-induced KTD.

CA haplotype (−24C, 1249A) of *ABCC2* was associated with tenofovir-induced KTD, whereas TG was a protective haplotype (Table 5). Izzedine et al [13] reported the role of CATC haplotype (−24C, 1249A, 3563T, 3972C) of *ABCC2* in KTD. However, 3563T did not play such role in this haplotype analysis, because the prevalence of 3563T is 0% in the Japanese, according to the HapMap data, and haplotype with only −24C plus 1249A still exhibited its effect on tenofovir-induced KTD (Table 5; www.hapmap.org). The reported association between tenofovir-induced KTD and 526G and 2759C of *ABCC10* described by Pushpakom et al [21] was also not reproduced in this study. Furthermore, SNPs in *ABCC4*, *SLC22A6*, and *ABCB1* investigated in the present study did not show a significant association with tenofovir-induced KTD (Table 3).

Three main aspects of our study are important. First, this is the first study to our knowledge that elucidated the effect of SNPs on tenofovir-induced KTD conducted in a country other than European countries or the United States. Our study examined Japanese patients of genetic background different from patients of previous studies, which consisted mostly of whites. While SNPs −24C and 1249A of *ABCC2* have been speculated to correlate with tenofovir-induced KTD in previous studies, the present study confirmed that these SNPs are risk factors for tenofovir-induced KTD in nonwhites.

The result that the SNPs in *ABCC2* are a risk for tenofovir-induced KTD can also be applied to patients with other genetic backgrounds who host SNPs −24C and 1249A. Notably, the impact of SNPs on tenofovir-induced KTD might be more significant in Africans and Indians than in Japanese or whites, considering that the allele frequencies of −24C and 1249A are higher in these population according to the HapMap data (−24C; Africans 96.9%, Indians 92.6%, Japanese 80.8%, whites 81.9%, 1249A; Africans 21.7%, Indians 30.7%, Japanese 8.9%, whites 23.7%; www.hapmap.org).

Second, the study was designed to evaluate the exclusive effect of SNPs on tenofovir-induced KTD by excluding

Table 5. Association Between Haplotype in *ABCC2* and *ABCC4* and Kidney Tubular Dysfunction

SNP Marker/Haplotype	Allele	Allele/Haplotype Frequency, %		OR (95% CI)[a]	*P* Value
		KTD Group (n = 19)	Control Group (n = 171)		
ABCC2					
−24 C → T	C	97.4	78.4	10.22 (1.658–419.8)	.003
1249 G → A	A	28.9	12.3	2.91 (1.345–6.296)	.011
ABCC2 haplotype	CA	28.9	12.3	2.91 (1.295–6.221)	.011
	TG	2.6	21.6	0.098 (.002–.603)	.003
ABCC4					
559 G → T	T	21.1	12.3	1.905 (.705–4.614)	.213
4976 T → C	T	48	55.3	0.746 (.375–1.470)	.399
ABCC4 haplotype					
TT	TT	17.6	7.9	2.497 (.902–6.949)	.077

Abbreviations: CI, confidence interval; KTD, kidney tubular dysfunction; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] ORs and *P* values are for comparisons of allele/haplotype frequencies between the kidney tubular dysfunction and control groups.

possible predisposing factors for KTD, for example, active infection, malignancies, diabetes mellitus, and preexisting renal impairment, which are known risks for KTD [35]. Patients who showed no HIV-1 viral suppression were also excluded. Furthermore, the enrolled patients were Japanese only, and this helped to examine a study population with comparatively similar genetic background. The study population was also on the same antiretroviral regimen (ritonavir-boosted darunavir plus tenofovir/emtricitabine), and this also helped to evaluate more precisely the effect of SNPs, because plasma concentration of tenofovir is affected by concomitant antiretrovirals and the delta change in plasma tenofovir concentration likely differs in the presence of each concomitant drug [26].

Third, SNPs were examined in 190 patients in this study. To our knowledge, the number of enrolled patients is the largest among the studies that have so far examined the effect of SNPs on tenofovir-induced KTD. Thus, this feature provided the study a higher statistical power than previous studies.

Why are polymorphisms in *ABCC2* a risk for tenofovir-induced KTD, even though it is controversial whether MRP2 plays a role in the excretion of tenofovir via the luminal membrane? [18, 20] The exact mechanism has not been determined yet, but we speculate 2 hypotheses. First, there might be unknown endogenous substances that influence tenofovir nephrotoxicity in renal tubular cells, and SNPs in *ABCC2* modulate the function or transportation of such substances [15]. Second, MRP2 may indeed take part in transporting tenofovir, because various substances including methotrexate are reported to be a substrate of MRP2, and *ABCC2* mutation alters excretion of those substances [36, 37]. Further studies are warranted to elucidate the exact mechanism of these SNPs on tenofovir-induced KTD. Furthermore, the impact of these SNPs on KTD with long-term TDF use needs to be evaluated in prospective studies.

Several limitations need to be acknowledged. First, not all polymorphisms in genes of the targeted transporter proteins were examined. Thus, we might have missed other important SNPs on the function of tenofovir transportation. There might be other unknown transporter proteins for tenofovir excretion in the kidney that contribute to susceptibility to tenofovir-induced KTD as well. Second, the diagnostic criteria for TDF-induced KTD are not uniformly established in the field and are different in the published studies. The criteria applied in this study are not entirely similar to the ones used in previous studies that examined the role of SNPs in tenofovir-induced KTD. However, by excluding other predisposing factors for KTD and enrolling a large number of patients, this study succeeded in providing a clear-cut association between SNPs and tenofovir-induced KTD.

In conclusion, the present study demonstrated that SNPs in *ABCC2* associate with tenofovir-induced KTD in Japanese patients, in a setting that excluded other predisposing factors. Assessment of renal tubular function is more cumbersome and costly to monitor than serum creatinine. However, monitoring tubular function is clinically important, because undetected long-term tubular dysfunction might lead to premature osteopenia due to phosphate wasting and accelerated progression of renal dysfunction. Close monitoring of tubular function is warranted in patients with *ABCC2* −24C and 1249A under TDF treatment.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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INTERNAL MEDICINE

CASE REPORT

Drug-Induced Acute Interstitial Nephritis Mimicking Acute Tubular Necrosis after Initiation of Tenofovir-Containing Antiretroviral Therapy in Patient with HIV-1 Infection

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Abstract

We describe a case of 68-year-old Japanese man with HIV-1 infection who developed acute kidney injury with prominent tubular dysfunction immediately after starting tenofovir-containing antiretroviral therapy. Antiretroviral therapy was discontinued in two weeks but renal function, as well as tubular function, did not shown full recovery even at a 3-year follow-up examination. Acute tubular necrosis, a rare but well-known side effect of tenofovir, was suspected, but kidney biopsy confirmed interstitial nephritis. It is important to distinguish drug-induced interstitial nephritis from acute tubular necrosis, because early steroid administration can improve renal dysfunction caused by acute interstitial nephritis.

Key words: tenofovir, acute interstitial nephritis, acute tubular necrosis, acute kidney injury, HIV infection, kidney biopsy

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Introduction

Renal proximal tubular dysfunction is a well-known side effect of tenofovir (1, 2). Although rare, it sometimes leads to acute tubular necrosis (ATN) and results in acute kidney injury (AKI) (1, 3). Drug-induced acute interstitial nephritis has a similar clinical presentation to ATN, but has different etiology and management (4, 5). Here we report a case of tenofovir-induced acute interstitial nephritis (AIN) which mimicked ATN after initiation of tenofovir-containing antiretroviral therapy (ART).

Case Report

A 68-year-old Japanese man with history of hypertension and diabetes mellitus was diagnosed with HIV infection and pneumocystis pneumonia (PCP). The latter was treated with sulfamethoxazole/trimethoprim plus prednisolone for three weeks, and the patient was referred to our hospital. Reactivation of PCP occurred and he was again treated with sulfamethoxazole/trimethoprim for three weeks. After completion of PCP treatment, sulfamethoxazole/trimethoprim was replaced with atovaquone for secondary prophylaxis, and one month later ART was started with tenofovir/emtricitabine plus lopinavir/ritonavir (baseline CD4 count 39/μL, HIV viral load 990,000 copies/mL). Baseline renal function tests were within the normal range (serum creatinine 0.53 mg/dL, blood urea nitrogen 8.7 mg/dL) with urine β2 microglobulin (β2MG) of 2,327 μg/L. The concurrent drugs were atovaquone (which was switched to prophylactic dose of sulfamethoxazole/trimethoprim on ART day 2), azithro-

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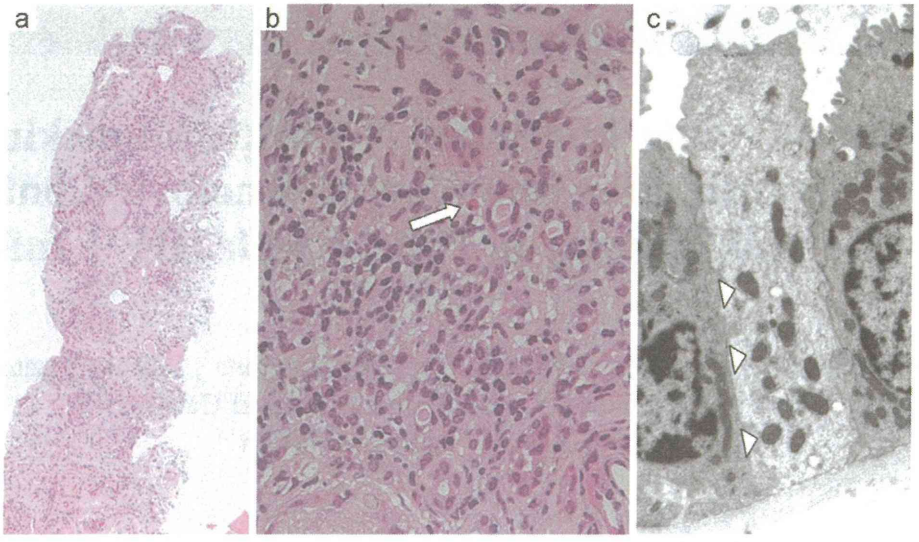

Figure 1. The microscopic findings in the renal biopsy specimen. (a) Diffuse interstitial inflammation with histologically normal glomeruli (Hematoxylin and Eosin (H&E) staining, ×10). (b) Prominent interstitial inflammatory infiltrates characterized by lymphocytes, plasma cells, and focal eosinophils (white arrow) (H&E staining, ×400). (c) Electron microscopic examination showed mitochondria normal in size and morphology in proximal tubular epithelial cells (white arrow heads) (×5,000).

mycin 1,200 mg/week, and olmesartan. No concurrent nonsteroidal anti-inflammatory drug was used.

Serum creatinine started to rise and on ART day 14, it reached 2.66 mg/dL with β2MG of 321,400 μg/L. No fever or rashes were observed, but prominent eosinophilia was noted (18.6% of leukocytes, 4,400/μL). Urine dipstick test showed proteinuria +3, occult blood +2, and glycosuria +1, together with renal tubular epithelial cells and granular casts in urine. Serum potassium, sodium, and phosphate levels were within the normal ranges. Serum IgE was high (1,040 IU/mL), and serum antinuclear antibodies, antineutrophil cytoplasm antibody, and cryoglobulin were negative. Renal ultrasonography was also negative for specific findings.

ART and the other concurrent medications, with the exception of azithromycin, were discontinued on that day. Hydration with central venous catheter was started. At 21 days after commencement of ART, serum creatinine reached a peak level of 5.39 mg/dL, though renal function started subsequently to improve slowly. At 32 days after discontinuation of ART, ART with darunavir/ritonavir plus raltegravir was provided (serum creatinine 2.59 mg/dL). The patient was discharged 44 days after re-commencement of ART with a CD4 count of 247/μL, and HIV viral load of 2,700 copies/mL. Within 3 months after discharge, HIV viral load was suppressed to <50 copies/mL with a CD4 count of 316/μL.

Five months after the episode, renal biopsy was performed (serum creatinine 1.76 mg/dL, β2MG 15,677 μg/L). Examination of the specimen showed interstitial infiltration of lymphocytes, plasma cells, and a few eosinophils. There was no vacuolation in tubular cells and the brush border was intact. The glomeruli were histologically normal (Fig. 1a, b). Immunofluorescence study was negative for IgG, IgM, IgA, C1q, C3, C4, or fibrinogen. Electron microscopic examination demonstrated no abnormalities in the mitochondria of tubular cells (Fig. 1c). The final diagnosis was drug-induced AIN. Serum creatinine and β2MG were still elevated three years later at 1.47 mg/dL and 25,718 μg/L, respectively.

Discussion

We described a case of tenofovir-induced AIN, which clinically mimicked ATN, after commencement of tenofovir-containing ART. Although the causative drugs were discontinued in two weeks, renal function did not show full recovery and the patient developed chronic kidney disease (Fig. 2). Tenofovir was highly likely the causative drug, because sulfamethoxazole/trimethoprim, the other drug which was used just before the occurrence of AIN, had been intermittently used for more than two months before the introduction of ART without any complications. To our knowledge, this is the fourth reported case of tenofovir-induced AIN, in addition to the three cases reported by Schmid et al. (6). Nevertheless, it is difficult to entirely role out the involvement of sulfamethoxazole/trimethoprim in occurrence of this AIN case. A combination effect of TDF and sulfamethoxazole/trimethoprim might have played a role.

It is difficult to diagnose interstitial nephritis based on clinical and laboratory findings only, and renal biopsy is required for a definitive diagnosis (4, 5). Only 5 to 10% of patients present with the classic triad of AIN symptoms: fever, rash, and eosinophilia (4, 5). However, renal biopsy is not performed in many cases with tenofovir-induced renal dysfunction, and thus, a considerable number of tenofovir-

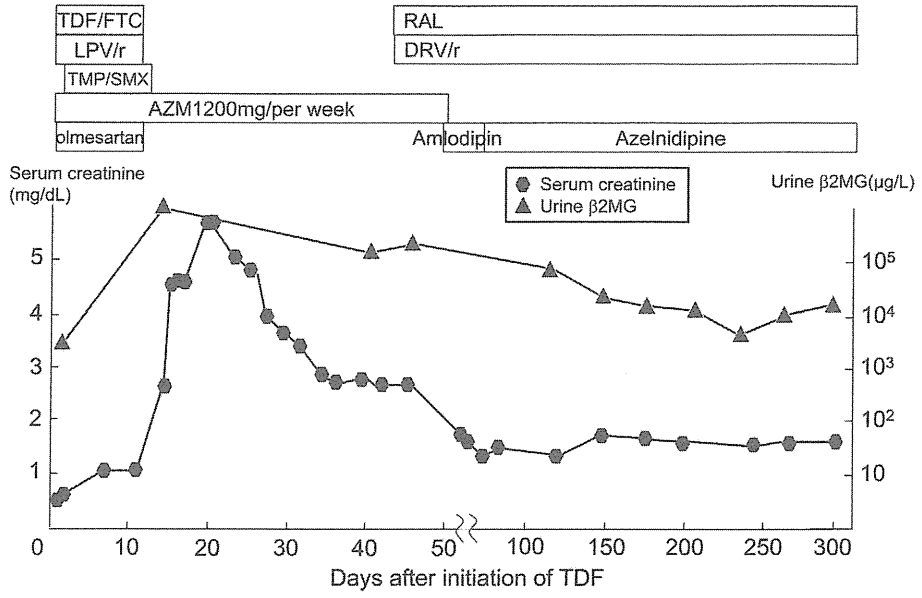

Figure 2. The clinical course of the patient. TDF/FTC: tenofovir/emtricitabine, LPV/r: ritonavir-boosted lopinavir, RAL: raltegravir, DRV/r: ritonavir-boosted darunavir, TMP/SMX: trimethoprim/sulfamethoxazole, AZM: azithromycin, β2MG: β2 microglobulin

induced AIN may have been misdiagnosed. Although a prominent eosinophilia and hyper-IgE (1,040 IU/mL) was noted for this case, these laboratory findings are commonly observed in patients with HIV-1 infection (7, 8). It is therefore difficult to dignose AIN solely based on these laboratory findings in patients with HIV infection.

The pathomechanism of tenofovir-induced ATN is considered to be mitochondrial toxicity in proximal tubular cells (9, 10). In contrast, interstitial nephritis occurs as an allergic response triggered by exposure to a drug (4, 5). It is important to distinguish AIN from ATN, because early steroid administration can improve the recovery of renal function in AIN (4, 5).

AIN should always be included in the differential diagnosis in a patient with AKI and prominent renal tubular damage following the introduction of tenofovir. In addition to prompt discontinuation of tenofovir, renal biopsy followed subsequently with steroid therapy at an early stage could produce a favorable renal outcome.

Author's disclosure of potential Conflicts of Interest (COI).
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CASE REPORT

Long-term control of CMV retinitis in a patient with idiopathic CD4$^+$ T lymphocytopenia

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Abstract Cytomegalovirus (CMV) retinitis with idiopathic CD4$^+$ T lymphocytopenia (ICL) is rare and difficult to control. We report a first case for long-term control of CMV retinitis with ICL using interleukin-2 (IL-2) therapy and succeeded in discontinuation of anti-CMV therapy. A 49-year-old Japanese woman was diagnosed with ICL based on low CD4$^+$ count (72/μl), negative for HIV-1 and -2 antibodies, and absence of any defined immunodeficiency diseases or immunosuppressive therapy. PCR test of the aqueous humor in the right eye was suggestive of CMV retinitis. She was treated with systemic ganciclovir, but after several relapses of CMV retinitis, rhegmatogenous retinal detachment appeared in the right eye and she became blind in that eye. Three years later, she developed CMV retinitis in the left eye. Although she received systemic and focal anti-CMV treatments, the retinitis showed no improvement. Finally, retinal detachment occurred, and she underwent vitrectomy. IL-2 was injected to increase CD4$^+$ counts. Because of hyperpyrexia, blepharedema, central scotoma, and color anomaly, we changed to low-dose IL-2 therapy with no side effects. Finally, we succeeded in increasing the CD4$^+$ count to more than 200/μl after discontinuation of low-dose IL-2 therapy. CMV retinitis never recurred after discontinuation of anti-CMV therapy, with good visual acuity of 20/20 in the left eye. She developed blindness of the first affected right eye, whereas the visual acuity of the left eye remains excellent more than 12 years after the onset of CMV retinitis through the combined use of anti-CMV therapy, IL-2 therapy, and vitrectomy.

Keywords Cytomegalovirus retinitis · CD4$^+$ T lymphocytopenia · IL-2 · Vitrectomy

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Introduction

Idiopathic CD4$^+$ T lymphocytopenia (ICL) was first reported in 1992 as a new disease entity [1, 2]. The syndrome encompasses patients with (1) absolute CD4$^+$ T lymphocyte (CD4$^+$) counts less than 300/μl or less than 20 % of total T cells on more than one occasion, (2) no evidence of human immunodeficiency virus (HIV)-1 or -2 infection, and (3) absence of any defined immunodeficiency diseases or treatment that lowers CD4$^+$ counts.

Opportunistic infections similar to those associated with HIV infection are often encountered in patients with ICL. However, only a few patients with ICL complicated by cytomegalovirus (CMV) retinitis have been reported [3–5]. In this report, we describe a case of CMV retinitis in both eyes in a patient with ICL; vision was completely lost in

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one eye, whereas good vision was successfully maintained in the other eye by anti-CMV therapy, IL-2 therapy, and vitrectomy.

Case report

A 49-year-old Japanese woman visited her family physician in 1994 with visual field impairment in the right eye. She had no special family history, and reported having herpes zoster infection when 40 years old. Fundus examination of the right eye showed hemorrhagic retinitis with exudates in the inferior aspect of the optic disc, and polymerase chain reaction (PCR) test of the aqueous humor from the anterior chamber was suggestive of CMV retinitis. Leukocyte count was 5,290/μl, with a total lymphocyte count of 332/μl (5.6 %). Both $CD4^+$ and $CD8^+$ T lymphocyte ($CD8^+$) counts were low, at 91/μl (27.6 %) and 72/μl (21.8 %), respectively. Antibodies for HIV-1 and -2 were negative. Her general condition was satisfactory except for CMV retinitis; the patient had no other diseases such as cancer and was taking any immunosuppressant. Based on the foregoing findings, the final diagnosis was ICL.

The patient was treated with systemic ganciclovir but experienced several relapses of CMV retinitis. Rhegmatogenous retinal detachment appeared in the right eye in 1997. Retinal detachment surgery (retinal backing, encircling, and cryopexy) was successful with reattachment of the retina. However, CMV retinitis recurred in the right eye, which resulted in blindness in 1997.

Three years later, she developed CMV retinitis in the left eye. Although she received systemic and focal anti-CMV treatments (systemic ganciclovir, vitreous injection of ganciclovir and foscarnet, and replacement of the vitreous ganciclovir implant), the retinitis showed no improvement but rather gradual extension. Accordingly, she was referred to the National Center for Global Health and Medicine in September 2001. The main clinical course after the first visit is shown in Fig. 1. At arrival, she had already developed phthisis bulbi of the right eye. The visual acuity of the left eye was 20/20, and no inflammatory cells were detected in the anterior chamber. The ganciclovir implant was seen in the inferotemporal vitreous; however, CMV retinitis with granular border was noted in the inferotemporal retina. No other general abnormal findings were evident except for blood tests: leukocyte count of white cells 3,010/μl, total lymphocyte count 400/μl, $CD4^+$ count 164/μl, and $CD8^+$ count 43/μl. Intravenous injection of cidofovir resulted in significant improvement of CMV retinitis, but the condition recurred after 4 months. Finally, retinal detachment occurred in the left eye (Fig. 2). She underwent vitrectomy in April 2002 and the retina was reattached. During this period, she also developed a subcutaneous cryptococcus abscess in the left thigh. The abscess had disappeared subsequent to surgical

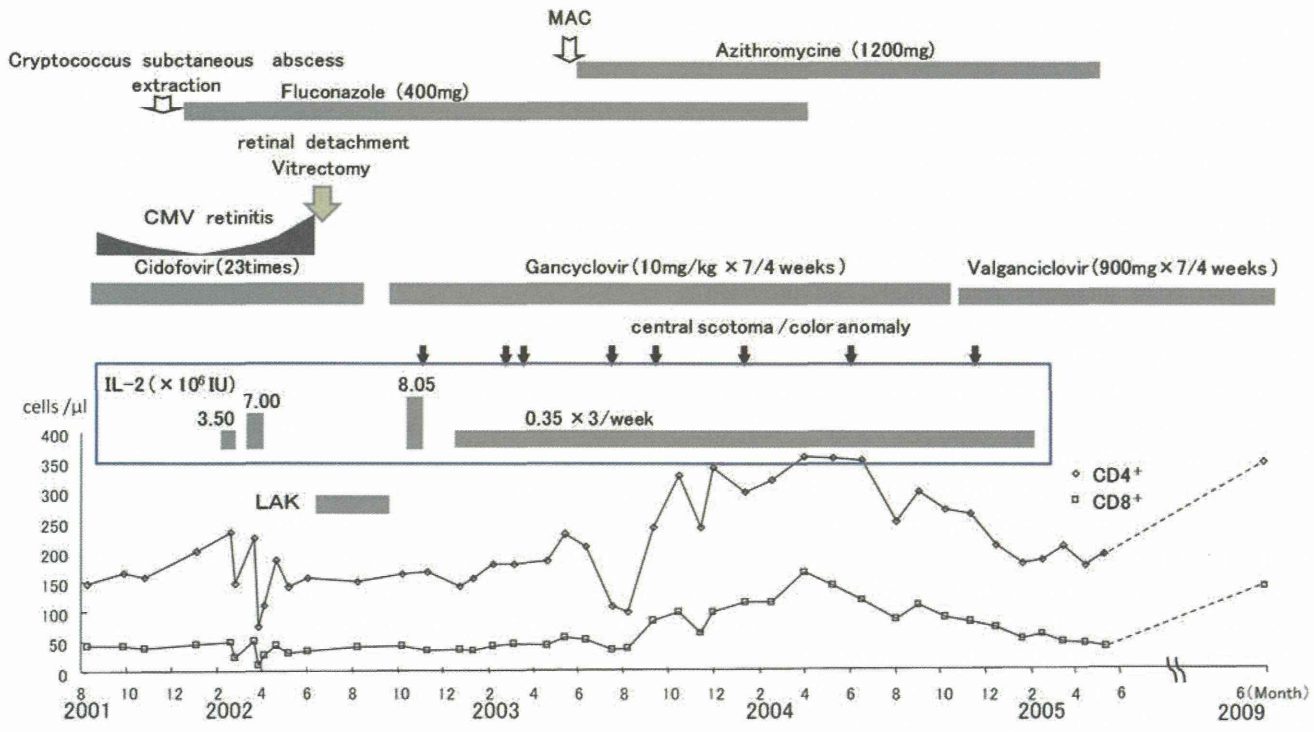

Fig. 1 Clinical course

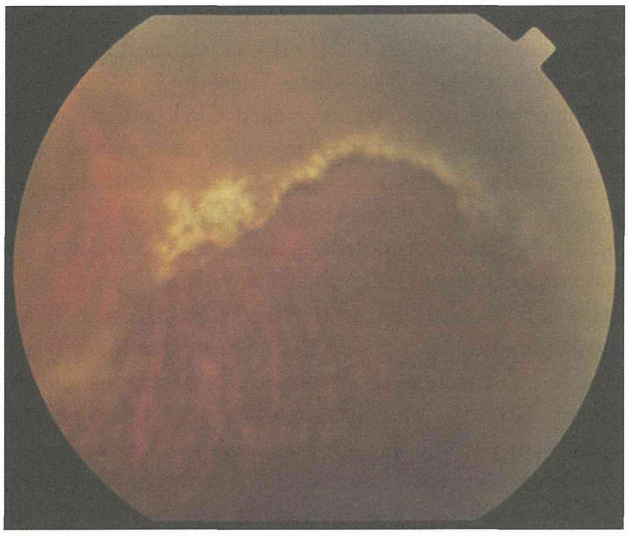

Fig. 2 Retinal detachment in the left eye: the retina under the white granular border is already healed of cytomegalovirus (CMV) retinitis with tight atrophic adhesion. The peripheral healthy retina over the border is progressing toward retinal detachment

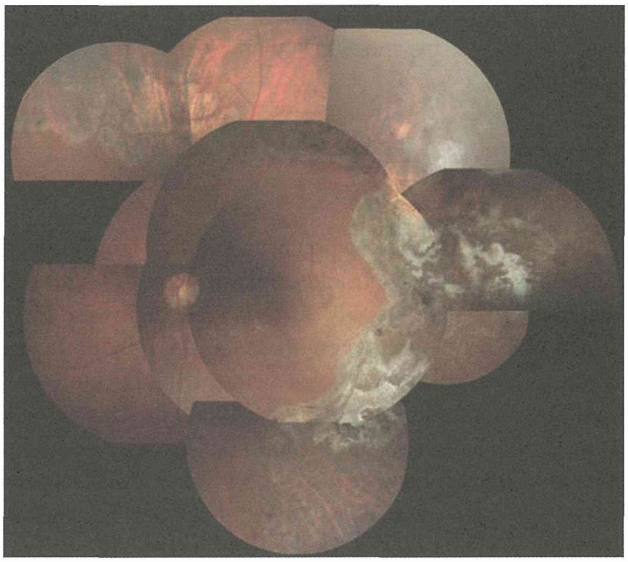

Fig. 3 Retina reattached after vitrectomy: the optic nerve and macula are intact. The atrophic retina after CMV retiniti presents outside the arcade vessel with laser scar and proliferative epiretinal membrane. There is no relapse of CMV retinitis after vitrectomy

extraction and oral fluconazole (400 mg) by April 2004. According to ethics standard, the institutional review board permitted starting interleukin-2 (IL-2) therapy to increase the immune level. Subcutaneous IL-2 injection was started with written patient's informed consent in February 2002 (the first dose was 350,000 IU × 2 for 5 days, total 3,500,000 IU; and the second dose was 700,000 IU × 2 for 5 days, total 7,000,000 IU) to increase $CD4^+$ count, but the count decreased further to 37/μl. To improve the immune status, lymphokine activated killer cells (LAK) therapy was adopted four times from June to August 2002. However, clinical improvement was not observed, and $CD4^+$ count hovered around 40–50/μl. Therefore, a third dose of IL-2 (1,050,000 IU × 2 for 5 days) was scheduled in November 2002. Four days after subcutaneous injection, she developed hyperpyrexia, blepharedema, central scotoma, and color anomaly. Accordingly, we provided a decreased dose of IL-2 for 700,000 IU on the last day (total dose of 8,050,000 IU) and stopped the therapy. The Goldmann perimeter did not show central scotoma, but a pseudo-isochromatic plate showed color deficiency. Because the complications of central scotoma and color deficiency disappeared several days after each IL-2 injection, we restarted low-dose IL-2 (350,000 IU three times a week) in February 2005.

Mycobacterium avium complex (MAC) infection was suspected based on the findings of chest computed tomography and gastric juice culture positive for the acid-fast bacteria in June 2003. Prophylactic treatment with azithromycin (1,200 mg/day) was continued until June 2005 and successfully discontinued. In terms of anti-CMV treatment, intravenous ganciclovir (480 mg/day) was administered from September 2002 instead of cidofovir injection (total 23 times), and the dose was adjusted according to the severity of bone marrow suppression, together with IL-2 therapy. With the gradual increase in $CD4^+$ count, we were able to change the anti-CMV therapy from intravenous ganciclovir to oral valganciclovir (900 mg/day) in December 2004, and tapered the dose of valganciclovir (1,800 mg × 7 days/month). The $CD4^+$ count has been above 200/μl without IL-2 therapy since 2005. Valganciclovir was discontinued in June 2009.

Since the vitrectomy in 2002, CMV retinitis has not recurred (Fig. 3), and the patient continued to have good visual acuity of 20/20 in the left eye when tested in the last follow-up examination in January 2012.

Discussion

Many ICL cases with various opportunistic infections similar to those of acquired immunodeficiency syndrome (AIDS) patients have been reported since 1989. However, this disease is not well known in the field of ophthalmology because few cases of CMV retinitis have been reported. In this case, the patient was diagnosed with ICL based on low $CD4^+$ count (< 300/μl), negativity for HIV, and absence of any defined immunodeficiency diseases or immunosuppressive therapy.

The etiology of ICL is not clear at present. However, the following mechanisms have been proposed. (1) Viral infection: Guputa et al. [6] reported the presence of

retroviral particles and found a human intracisternal A-type retroviral particle. These reports suggest that a virus other than HIV is involved in ICL. (2) Autoimmune disease. (3) Apoptotic depletion of $CD4^+$ cells associated with over-expression of Fas and Fas-ligand. The pathophysiology of ICL is thought to involve reduced cell-mediated immunity subsequent to low $CD4^+$ count. Therefore, the main complications associated with ICL are opportunistic infections and malignancies. Thus, this case developed CMV retinitis, subcutaneous cryptococcus infection, and MAC infection.

Bone marrow transplantation is the ultimate treatment for ICL. However, our patient had already suffered CMV retinitis, and we considered it was difficult to justify further suppression of the immune system before transplantation. The main treatment for ICL is designed to treat opportunistic infection or malignancy. Additionally IL-2 has stimulatory effects on T cells, leading to an increase in $CD4^+$ count; therefore, successful treatment of ICL with IL-2 has been reported [7–11]. The initial dose varies (38,000–600,000 IU/day, or 50,000 IU/m^2 weekly), and clinical response appears after approximately 5–6 months. Many side effects, mostly IL-2 dose dependent, have been reported, including hyperpyrexia, edema caused by blood vessel hyperpermeability, renal and hepatic dysfunction, and mental disorders. Our patient complained of central scotoma and color deficiency, as well as hyperpyrexia and blepharedema, which have not been reported in the past. We decided not to discontinue IL-2 therapy but to use low-dose IL-2. Finally, we succeeded in increasing the $CD4^+$ count without complications. The etiology of central scotoma and color anomaly remains unknown, but we presume they are related to hyperpermeability of blood vessels adjacent to the optic nerve, which resulted in reversible damage of the optic nerve.

It is thought there are two subtypes of ICL in terms of presence or absence of $CD8^+$ T lymphocytopenia, and low $CD8^+$ count at diagnosis represents a subset of ICL with a worse prognosis and increased risk for a serious opportunistic infection or death [12]. Although this case showed a low $CD8^+$ count, the left eye has maintained good visual acuity of 20/20 for more than 12 years from the onset of CMV retinitis.

What are the reasons for success and the mechanism of the longitudinal remission in the left eye? One can presume that the long-term use of anti-CMV therapy played a role; we used anti-CMV therapy for more than 15 years from the onset of ICL, and continued the use of low-dose valganciclovir for 7 years after the remission of CMV retinitis in the left eye to prevent its recurrence. The availability of oral anti-CMV therapy (valganciclovir) made it possible to use the drug over such a long term.

What about IL-2 therapy? The $CD4^+$ count greatly decreased in the induction period of IL-2 therapy in this case. Moreover, Zonios et al. [13] reported one-fifth of the patients resolving their lymphocytopenia within 3 years of diagnosis, which suggests the time course is spontaneous remission. On the other hand, transient decrease in IL-2-responsive lymphocytes is reported after initiation of IL-2 infusion [13]. Cunningham-Rundles et al. [7] have described the delayed enhancement of proliferation is the result of new clonal T cell populations permitted to emerge and expand. IL-2 is also well known to expand peripheral natural killer cell numbers and eosinophilia. Although a single case does not provide definitive answers, adjunctive therapy with IL-2 could trigger not quantitative but functional immune recovery of $CD4^+$ over the long term and allowed discontinuation of anti-CMV therapy.

Finally, vitrectomy was performed to repair retinal detachment. Vitrectomy has been thought to be effective for inflammatory activity and adjunctive medical therapy [14]. In this case, the assumption is that vitrectomy plays a role in the mechanical removal of CMV and inflammatory cytokines, and facilitates diffusion of anti-CMV drugs in the vitreous, which prevents the recurrence of CMV retinitis.

ICL is still an uncommon disease, especially for ophthalmologists; however, precise diagnosis and early treatment can facilitate longitudinal remission of opportunistic infection.

Conflict of interest None of the authors has conflict of interest with the submission.

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ARTICLE

A double-blind comparative study of the safety and efficacy of caspofungin versus micafungin in the treatment of candidiasis and aspergillosis

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Abstract The safety and efficacy profile of caspofungin and micafungin in Japanese patients with fungal infections were directly compared in this prospective, randomized, double-blind study. The proportion of patients who developed significant drug-related adverse event(s) (defined as a serious drug-related adverse event or a drug-related adverse event leading to study therapy discontinuation) was compared in 120 patients [caspofungin 50 mg, or 50 mg following a 70-mg loading dose on Day 1 (hereinafter, 70/50 mg) group: 60 patients; micafungin 150 mg: 60 patients]. The overall response rate was primarily evaluated in the per-protocol set (PPS) population. The proportion of patients who developed significant drug-related adverse events was 5.0 % (3/60) in the caspofungin group and 10.0 % (6/60) in the micafungin group [95 % confidence interval (CI) for the difference: −15.9 %, 5.2 %]. The favorable overall response in the PPS population for patients with esophageal candidiasis, invasive candidiasis, and chronic pulmonary aspergillosis including aspergilloma was 100.0 % (6/6), 100.0 % (3/3), and 46.7 % (14/30) in the caspofungin group, and 83.3 % (5/6), 100.0 % (1/1), and 42.4 % (14/33) in the micafungin group, respectively. In Japanese patients with *Candida* or *Aspergillus* infections, there was no statistical difference in the safety between caspofungin and micafungin. Consistent with other data on these two agents, the efficacy of caspofungin and micafungin was similar.

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Introduction

The importance of deep-seated fungal infections in Japan is considered to be increasing due to the rise in the number of immunocompromised patients associated with the introduction of advanced medical treatment and the aging of the Japanese population as a whole. *Candida* spp. and *Aspergillus* spp. are the most important causative pathogens in Japan, the same as in other countries [1, 2].

Echinocandins inhibit the biosynthesis of (1,3)-β-D-glucan, the structural component of fungal cell wall, thereby, exhibiting antifungal activity against *Candida* spp. and *Aspergillus* spp. Although caspofungin, micafungin sodium (hereinafter, micafungin), and anidulafungin have been approved and are used worldwide, micafungin is the only approved echinocandin antifungal agent in Japan at the time of this study.

Caspofungin has been shown to be effective as the primary therapy for esophageal candidiasis and invasive candidiasis, as salvage therapy for invasive aspergillosis, and as empirical therapy in patients with persistent fever and neutropenia. To date, caspofungin has been approved for use in over 80 countries worldwide, including the United States and Europe [3–6]. A comparator-controlled study of caspofungin and micafungin conducted in patients with candidemia has been reported by Pappas et al. In this study, micafungin 100 mg or 150 mg once daily was shown to be effective (non-inferior) compared to caspofungin 50 mg daily following a 70-mg loading dose on Day 1 [7]. Additionally, in a cohort analysis, caspofungin and micafungin were compared as empirical therapy in patients with febrile neutropenia, with similar efficacy reported [8]. There are no reports on the comparative study of caspofungin and micafungin for aspergillosis.

Herein, we report the results of a randomized, double-blinded, comparative study of caspofungin versus micafungin conducted in Japanese patients with *Candida* or *Aspergillus* infections. The safety and efficacy profiles of caspofungin and micafungin were compared.

Study patients and study plan

Objective and study design

This is a randomized, multicenter, double-blind, comparative study. The study was conducted in 43 study sites in Japan from August 2008 through July 2010. The protocol was reviewed by the Institutional Review Board of each participating site, and written informed consent was obtained from each patient. The protocol was also registered on clinicaltrials.gov (NCT00717860). In this study, a serious drug-related adverse event or a drug-related adverse event leading to study therapy discontinuation was defined as significant drug-related adverse event(s). Definitions of adverse events and drug relationships, and the determination of seriousness basically complied with the "Definitions and Terminology Associated with Clinical Safety Experience" in the International Conference on Harmonisation (ICH)-E2 [9]. The primary objective of this study was to compare the difference in the proportion of patients who develop significant drug-related adverse events(s) between the caspofungin and micafungin groups. The secondary objective was to evaluate the difference in the overall response by each of esophageal candidiasis, invasive candidiasis, and aspergillosis.

Patient inclusion criteria

Japanese patients aged 20 years and over were enrolled in this study following obtainment of written informed consent. Patients who fulfilled the criteria indicated below were enrolled as probable disease cases. When causative fungi (*Candida* spp. or *Aspergillus* spp.) were identified by culture or relevant organisms with specific morphology (yeast or acutely branching mold with septated hyphae) were observed by microscopic examination in addition to the criteria below, then patients were enrolled as proven disease cases. Both probable and proven disease cases were the target population in this study.

Criteria for probable disease:

- Esophageal candidiasis: patients with clinical symptoms of esophageal candidiasis (i.e., odynophagia, dysphagia, and heartburn) and plaque observed on the esophageal mucosa by endoscopy.
- Candidemia: patients with fever >38 °C observed, or fever of ≥37.5 °C that continues for 1 h or more despite the use of antibiotic therapy and positive results for the (1,3)-β-D-glucan test.
- Other types of invasive candidiasis (except candidemia): fungal infection strongly suspected at screening based on the clinical course and symptoms, typical radiographic imaging findings on X-ray and computed tomography (CT) (based on infection site), and positive results for the (1,3)-β-D-glucan test.
- Invasive aspergillosis: patients with risk factors of fungal infections (e.g., neutropenia, immunosuppressive treatment), clinical symptoms (e.g., fever, generalized malaise, coughing, sputum, bloody sputum, dyspnea), characteristic radiographic imaging findings (e.g., infiltration shadow, nodular shadow, cavitary lesions, or halo sign), and positive results for *Aspergillus* galactomannan antigen (enzyme-linked immunosorbent assay).
- Chronic pulmonary aspergillosis (except pulmonary aspergilloma): patients with clinical symptoms (e.g.,

fever not responding to antibiotic agent, body weight decreased, wet coughing, bloody sputum), characteristic radiographic imaging findings (e.g., pericavity infiltration, increasing size of cavity, or fluid collection in the cavity), and positive results for *Aspergillus* antibody or *Aspergillus* galactomannan antigen.

- Pulmonary aspergilloma: patients who have clinical symptoms (e.g., sputum, bloody sputum, hemoptysis, fever, dyspnea, coughing), characteristic radiographic imaging findings (e.g., coccus image in the cavity, thickened cavity wall, pleural thickening, or fluid collection in the cavity), and positive results for *Aspergillus* antibody.

Of note, patients who received prior antifungal therapies (other than echinocandins) were also allowed to enroll in this study. In such cases, the patients were evaluated on whether they met the criteria of refractoriness (the patient received an antifungal agent within 7 days prior to study therapy administration, but the disease progressed or clinical improvement was not observed) or intolerance (there is a significant problem in tolerance during the administration of prior antifungal agents as judged by the investigators).

Patients who fall under any of the criteria listed below were to be excluded: patients with mycoses due to causes other than *Candida* spp. and *Aspergillus* spp.; patients who had already received caspofungin or micafungin for the current fungal infection within the 7 days prior to initiation of the study; International Normalized Ratio (INR) (prothrombin time) of >2 × ULN (upper limit of normal) for patients not receiving anticoagulants; INR >4 × ULN for patients receiving anticoagulants; total bilirubin of >5 × ULN; aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (ALP) of >5 × ULN; patients with a history of serious drug-related allergy or sensitivity; patients with moderate or severe hepatic insufficiency (acute hepatitis, hepatic cirrhosis, etc.); patients who received another investigational drug within 1 month prior to study entry; patients who are pregnant, intend to become pregnant during the period up to 2 weeks after study completion, or are lactating.

Treatment plan

The randomization was stratified by infection category [esophageal candidiasis, candidemia, other types of invasive candidiasis (except candidemia), invasive aspergillosis, chronic pulmonary aspergillosis, and pulmonary aspergilloma] using a random permuted block, with the caspofungin group and micafungin group allocated at a ratio of 1:1. Patients, study investigators, and the sponsor remained blinded to the treatment group throughout the study. The pharmacist or preparer of the study therapy at each site was not blinded to the treatment group, but this individual could not be involved with any evaluation or judgment of efficacy and safety in this study.

Each patient received intravenous administration of caspofungin (esophageal candidiasis: 50 mg, invasive candidiasis and aspergillosis: 70/50 mg) once daily or micafungin 150 mg once daily for approximately 1 h in a blinded fashion. The treatment periods were 7–28 days for patients with esophageal candidiasis, 14–56 days for patients with invasive candidiasis, and 14–84 days for patients with aspergillosis. Patients with esophageal candidiasis were treated with study therapy for at least 3 days after the resolution of clinical symptoms and signs. Patients with candidemia were treated for at least 14 days after the last positive culture result for *Candida* spp. Patients with aspergillosis were treated for at least 7 days after the resolution of clinical symptoms/signs and at least 14 days after the resolution of neutropenia (absolute neutrophil count; ANC: >500/μL). The use of other systemic antifungal agents and rifampin was prohibited until the time of the efficacy evaluation.

Safety and efficacy evaluation

With regard to the safety of the study drug, the investigators recorded all adverse events and drug-related adverse events occurring from the initiation of study therapy through 14 days after the last dose of the study drug, based on any abnormal physical findings, vital signs, and laboratory tests, including red blood cell count, white blood cell count, hemoglobin, hematocrit, platelet count, total protein, albumin, total bilirubin, direct bilirubin, AST, ALT, γ-glutamyl transpeptidase (γ-GTP), ALP, lactate dehydrogenase, blood urea nitrogen, creatinine, Na, K, Cl, Ca, uric acid, blood glucose, C-reactive protein, urinalysis, prothrombin time, and partial thromboplastin time. All safety information pertaining to a significant drug-related adverse event was reviewed by the Independent Safety Assessment Committee (ISAC) under blinded conditions for study therapy. In addition, with regard to hepatic function tests, maximum values from the study period were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 3 [10].

The diagnosis of patients enrolled into this study was reviewed by an Independent Efficacy Assessment Committee (IEAC). The efficacy results in this study were based on the overall response, which included the resolution or improvement of clinical symptoms and radiographic imaging findings [or eradication of *Candida* (microbiological response) in patients with candidemia]). All efficacy evaluations made by the investigators were reviewed by the IEAC in a blinded fashion, and the judgment by the IEAC was considered as the final result.

The efficacy evaluation in esophageal candidiasis was conducted 5–7 days after the end of study therapy. The

overall response was determined as "favorable" in patients with esophageal candidiasis if clinical symptoms and signs of *Candida* infections (odynophagia, dysphagia, and heartburn) resolved and follow-up endoscopy results indicated at least a two-grade improvement (or return to Grade 0) in the predefined criteria (Grades 0, 1/2, 1, 2, 3, and 4) [11]. The efficacy evaluation in invasive candidiasis was conducted at the completion of study therapy. The overall response was determined to be "favorable" in patients with invasive candidiasis if the clinical symptoms and signs of *Candida* infections were resolved and follow-up blood culture was negative (for patients with candidemia) or follow-up radiographic imaging findings were "improved" [for patients with other types of invasive candidiasis (except candidemia)]. The efficacy evaluation in aspergillosis was conducted at the completion of study drug. The overall response was determined to be "favorable" in patients with aspergillosis if the clinical symptoms and signs of *Aspergillus* infections were "improved" or "stable", and follow-up radiographic imaging findings were "improved" or "stable". However, if the clinical symptoms and signs and radiographic imaging findings were both "stable" in patients with aspergillosis, the overall response was determined to be "unfavorable".

Identification of fungus and drug sensitivity study

Fungus isolated in the study was sent to Mitsubishi Chemical Medience Corporation and the organism was identified to the species level. The susceptibility of all isolated *Aspergillus* spp. and *Candida* spp. to antifungal agents was measured according to the guidance for microdilution technique M38-A2 (*Aspergillus* spp.) [12] and M27-A3 (*Candida* spp.) [13] of the Clinical and Laboratory Standards Institute (CLSI).

Statistical analysis

The safety analysis population was the all patients as treated (APaT) population (all randomized patients who received at least one dose of study therapy). The incidence and its 95 % confidence interval (CI) by treatment groups were calculated for the primary endpoint, namely, the proportion of patients who developed significant drug-related adverse events (a serious drug-related adverse event or a drug-related adverse event leading to study therapy discontinuation). In addition, 95 % CIs for the difference in the incidence between treatment periods were calculated using the Miettinen and Nurminen method (1985). The study was not powered to show a statistically significant difference between treatment groups.

The primary efficacy analysis population was the per-protocol set (PPS) population. The PPS included any patient who was diagnosed as having *Candida* or *Aspergillus* infections by the IEAC and received an appropriate course of study therapy (at least 5 days for the treatment of esophageal candidiasis or invasive candidiasis or at least 7 days for the treatment of aspergillosis), and in whom the efficacy evaluation was conducted in accordance with the study protocol. For esophageal candidiasis and candidemia, patients were included in the PPS population only when *Candida* spp. was confirmed by culture test. In addition, a secondary efficacy analysis was also performed using the full analysis set (FAS) population to confirm the consistency of the results. The FAS included any patient who received at least one dose of study therapy and was diagnosed as having *Candida* or *Aspergillus* infections by the IEAC.

Patients whose overall response was determined as "unable to be judged" were excluded from the overall response in the PPS analysis. In the FAS analysis, "unable to be judged" patients were treated as "unfavorable". The proportion of patients with a favorable overall response and its 95 % CI were calculated by three disease types (esophageal candidiasis, invasive candidiasis, and chronic pulmonary aspergillosis including aspergilloma), as judged by the IEAC. The analysis methods, handling, and the identification of the patients to be excluded from the PPS population mentioned above were determined before the unblinding.

Results

Study patients and patient background

One hundred and twenty-one patients were randomized. The average age of the randomized patients at the time of enrollment was 69.1 years and the proportion of male patients (79.3 %) was greater than that of female patients (20.7 %). The average weight was 48.8 kg and patients who were refractory to or intolerant of prior antifungal agents accounted for approximately one-quarter of enrollment. There were no patients with human immunodeficiency virus (HIV) infection, allogeneic stem cell transplant, or graft versus host disease. Major risk factors observed in patients with esophageal candidiasis were diabetes mellitus (25.0 %) and malignant tumor (25.0 %). Major risk factors in patients with invasive candidiasis were diabetes mellitus (31.6 %) and malignant tumor (26.3 %). Major risk factors in patients with chronic pulmonary aspergillosis were pulmonary disorder (31.4 %), tuberculosis sequelae (24.3 %), diabetes mellitus (21.4 %), malignant tumor (8.6 %), and use of steroids (5.7 %). There was no statistical difference between the caspofungin group and the micafungin group for any demographic or baseline data (Table 1)

The breakdown of APaT, FAS, PPS and populations in this study and the reasons for the exclusion of patients from

Table 1 Patient demographics and background conditions (all randomized patients)

	Total		Caspofungin		Micafungin		*p*-value[a]
	n	(%)	*n*	(%)	*n*	(%)	
Randomized patients	121		61		60		
Sex							0.472
Male	96	(79.3)	50	(82.0)	46	(76.7)	
Female	25	(20.7)	11	(18.0)	14	(23.3)	
Age (years)							0.815
Mean	69.1		68.9		69.3		
Standard deviation	10.1		11.2		9.0		
Weight (kg)							0.476
Mean	48.80		49.56		48.01		
Standard deviation	11.61		10.75		12.47		
Refractoriness or intolerance to prior antifungal agents							0.884
Refractory	23	(19.0)	12	(19.7)	11	(18.3)	
Intolerant	5	(4.1)	2	(3.3)	3	(5.0)	
Primary therapy	93	(76.9)	47	(77.0)	46	(76.7)	
Underlying risks							0.478[c]
Diabetes mellitus	28	(23.1)	11	(18.0)	17	(28.3)	
Pulmonary disorder[b]	25	(20.7)	13	(21.3)	12	(20.0)	
Malignant tumor	22	(18.2)	13	(21.3)	9	(15.0)	
Tuberculosis sequelae	20	(16.5)	9	(14.8)	11	(18.3)	
Use of immunosuppressive drugs	5	(4.1)	1	(1.6)	4	(6.7)	
Use of steroids	5	(4.1)	2	(3.3)	3	(5.0)	
Neutrophil count <500/mm^3	4	(3.3)	2	(3.3)	2	(3.3)	
Thermal burn	1	(0.8)	1	(1.6)	0	(0.0)	

[a]Chi-square test (*t*-test for age and weight)

[b]Pulmonary disorder includes bronchiectasis, tuberculosis, chronic obstructive pulmonary disease, pulmonary fibrosis, and pulmonary bulla

[c]Based on the comparison of the proportion of patients who have at least one of the underlying risks between two treatment groups

each population is included in Fig. 1. One patient was excluded from the APaT population because blinding was not maintained for this patient. Thirteen patients who were diagnosed as having infections caused by pathogens other than *Aspergillus* spp. and *Candida* spp., based on the determination of the IEAC, were excluded from the FAS population. The most common reason for why patients were excluded from the FAS population and the PPS population was unconfirmed "positive culture" for esophageal candidiasis and invasive candidiasis (15 patients). Most of these excluded patients were with probable candidemia. Candidemia patients were allowed to start study therapy based on the positive (1,3)-β-D-glucan test and clinical symptoms, and, as a result, most of the culture results in these patients were demonstrated as negative. Patients who were not classified into diseases predefined in the study protocol (two patients with aspergillosis not classified) were also excluded from the PPS population. In addition, there were exclusions due to the use of prohibited concomitant drugs (one patient) and insufficient study therapy duration (four patients). There was no notable difference in the number of patients within each treatment group in any of the three analysis populations.

The average dosages in the APaT population were 51.0 mg/day and 149.7 mg/day in the caspofungin and micafungin groups, respectively. The average duration of study drug treatment in the APaT population was 28.7 (range 2–84) days and 33.6 (range 1–84) days in the caspofungin and micafungin groups, respectively. The accounting of patients by disease type is presented in Table 2.

Safety evaluation

The number of patients who reported drug-related adverse events is shown in the APaT population in Table 3. Drug-related adverse events were reported in 38.3 % and 41.7 % of patients in the caspofungin and micafungin groups, respectively. Serious drug-related adverse events were reported in two patients; both were in the micafungin group (AST and ALT increased in one patient and rash in the other patient).

Abnormal values in ALT, AST, and ALP (maximal levels), regardless of the drug relationship, were assessed in an exploratory fashion in accordance with CTCAE Version 3. The numbers of patients who had Grade 2 or higher ALT, AST, or ALP elevations (>2.5 × ULN) were 3, 4, and 5, respectively, in the caspofungin group, and 6, 5,

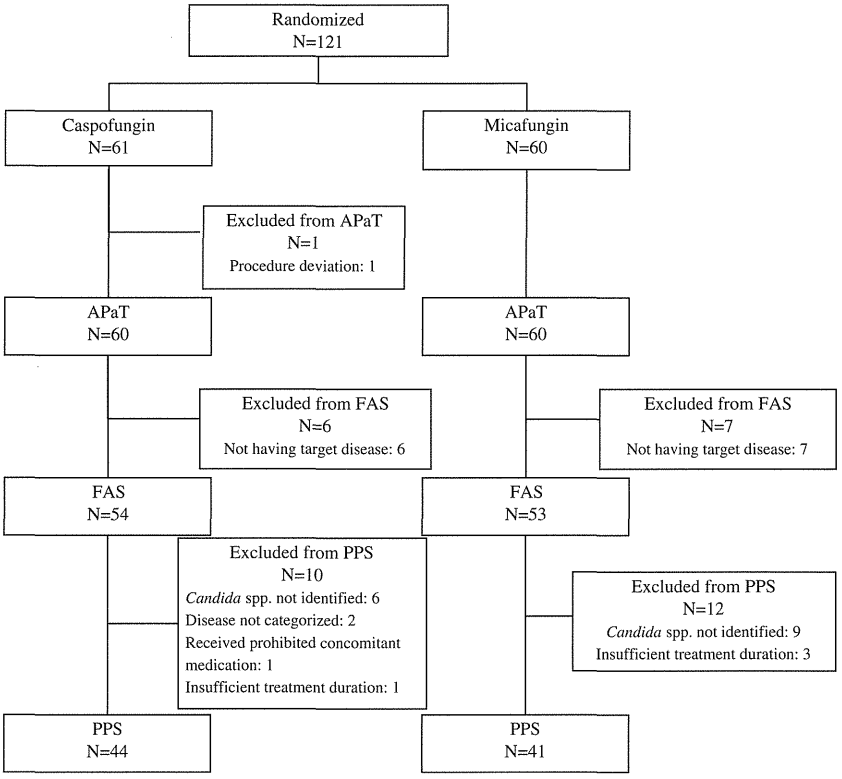

Fig. 1 Analysis populations and reasons for exclusion by treatment group. APaT: all patients as treated, FAS: full analysis set, PPS: per-protocol set

and 2, respectively, in the micafungin group. Of these, the number of patients who had Grade 3 ALT, AST, or ALP elevations (>5.0–20.0 × ULN) was 2, 3, and 1, respectively, in the micafungin group; none of the caspofungin-treated patients had Grade 3 elevations for ALT, AST, or ALP.

The proportion of patients fulfilling the primary endpoint of this study, the presence of one or more significant drug-related adverse events, was 5.0 % (95 % CI: 1.0, 13.9) in the caspofungin group and 10.0 % (95 % CI: 3.8, 20.5) in the micafungin group. The between-treatment difference was −5.0 % (95 % CI: −15.9, 5.2), thereby, showing no significant difference between the two groups. Significant drug-related adverse events were reported in three patients in the caspofungin group (all reported drug-related adverse events leading to study therapy discontinuation) and six patients in the micafungin group (two reported serious drug-related

Table 2 Disposition of patients by disease type

Disease type[a]	APaT		PPS	
	Number of patients [duration of therapy, mean days]		Number of patients	
	Caspofungin	Micafungin	Caspofungin	Micafungin
Esophageal candidiasis	9 [14.7]	7 [13.7]	8	6
Invasive candidiasis	9 [13.2]	9 [13.2]	3	1
Candidemia	6	7	1	0
Invasive candidiasis (excluding candidemia)	3	2	2	1
Aspergillosis	36 [37.1]	37 [42.1]	33	34
Invasive aspergillosis	1	0	1	0
Chronic pulmonary aspergillosis (including pulmonary aspergilloma)	33	37	32	34
Pulmonary aspergillosis (unclassified)	2	0	0	0
Other than mycosis[b]	6 [21.8]	7 [34.9]	0	0
Total	60 [28.7]	60 [33.6]	44	41

APaT all patients as treated, *PPS* per-protocol set

[a]Disease classification is based on the diagnosis by the Independent Efficacy Assessment Committee (IEAC)

[b]Other infectious diseases (not mycosis) diagnosed by the IEAC

Table 3 The number (%) of patients with clinical and laboratory drug-related adverse events (incidence ≥3 % in one or more treatment groups) [all patients as treated (APaT) population]

	Caspofungin[a]		Micafungin[b]	
	n	(%)	*n*	(%)
Patients in population	60		60	
With one or more drug-related adverse events	23	(38.3)	25	(41.7)
With one or more drug-related serious adverse events	0	(0.0)	2	(3.3)
Eye disorders	1	(1.7)	2	(3.3)
Gastrointestinal disorders	3	(5.0)	4	(6.7)
Constipation	0	(0.0)	2	(3.3)
Nausea	2	(3.3)	1	(1.7)
General disorders and administration site conditions	2	(3.3)	3	(5.0)
Injection site reaction	0	(0.0)	2	(3.3)
Hepatobiliary disorders	1	(1.7)	2	(3.3)
Infections and infestations	0	(0.0)	2	(3.3)
Laboratory abnormalities	14	(23.3)	18	(30.0)
Alanine aminotransferase (ALT) increased	5	(8.3)	4	(6.7)
Aspartate aminotransferase (AST) increased	6	(10.0)	3	(5.0)
Blood lactate dehydrogenase (LDH) increased	0	(0.0)	2	(3.3)
Blood potassium decreased	2	(3.3)	1	(1.7)
Blood potassium increased	1	(1.7)	3	(5.0)
Blood pressure increased	0	(0.0)	2	(3.3)
Eosinophil count increased	3	(5.0)	4	(6.7)
Gamma-glutamyl transpeptidase (γ-GTP) increased	2	(3.3)	2	(3.3)
Prothrombin time prolonged	2	(3.3)	0	(0.0)
White blood cell count decreased	1	(1.7)	2	(3.3)
White blood cell count increased	0	(0.0)	2	(3.3)
Platelet count increased	0	(0.0)	2	(3.3)
Blood alkaline phosphatase (ALP) increased	2	(3.3)	2	(3.3)
Nervous system disorders	3	(5.0)	2	(3.3)
Hypoesthesia	0	(0.0)	2	(3.3)
Skin and subcutaneous tissue disorders	1	(1.7)	6	(10.0)
Erythema	0	(0.0)	2	(3.3)
Rash	1	(1.7)	3	(5.0)
Vascular disorders	5	(8.3)	2	(3.3)
Hypertension	2	(3.3)	0	(0.0)
Phlebitis	2	(3.3)	2	(3.3)

[a]Patients with esophageal candidiasis received caspofungin 50 mg once daily. All other patients received caspofungin 50 mg once daily following a 70-mg loading dose on Day 1

[b]All patients received micafungin 150 mg once daily

Every patient is counted once for each applicable specific adverse event. A patient with multiple adverse events within a system organ class is counted once for that system organ class. A system organ class or specific adverse event appears in this table only if its incidence in one or more of the columns is greater than or equal to the percent incidence specified in the report title, after rounding

adverse events accompanied by study therapy discontinuation and four reported drug-related adverse events leading to study therapy discontinuation). The significant adverse events of three patients in the caspofungin group were elevation of ALP, AST, and γ-GTP, moderate rash, and elevation of AST and ALT. The significant adverse events in six patients of the micafungin group were elevation of AST and ALT, moderate rash, increased blood pressure level, occurrence of atrial fibrillation, elevation of γ-GTP alone, and elevation of AST, ALT, γ-GTP, ALP, and LDH with the occurrence of nausea. Nine patients in the caspofungin group and 10 patients in the micafungin group died during this study. None of the deaths were considered to be drug-related adverse events.

Efficacy evaluation

Of the 85 patients included in the PPS population, six patients were deemed to be "unable to be judged", and the favorable overall response rate was assessed for 79 patients. Favorable overall response rates in esophageal candidiasis, invasive candidiasis, and chronic pulmonary aspergillosis including aspergilloma are shown in Table 4. Among invasive candidiasis, one patient in the caspofungin group was candidemia and the others (two in caspofungin and one in micafungin) were peritoneal candidiasis patients. The overall response of caspofungin and micafungin in chronic pulmonary aspergillosis (other than aspergilloma) patients were