participating in this study were 71 years of age or older, and had both, clinical and neuroimaging, evidence of ischemic cerebral infarction. Only patients with symptom onset within 24 hours of admission were included in this study. Patients receiving a lipid-lowering agent were excluded. All subjects were systematically evaluated within 24 hours of symptom onset. Neurovascular evaluation, including underlying chronic condition assessment, serial neurological examinations including Glasgow Coma Scale, 12-lead electrocardiograms, and transthoracic echocardiograms, in addition to routine hematologic and chemistry profiles, was completed in all subjects. During this period, vital signs were measured at least every 2 hours, including noninvasive cuff BP, pulse rate, and body temperature, during the first 24 hours, and continued every 2 to 8 hours up to 72 hours. Antihypertensive medications was given at the discretion of physicians not involved in the study, who in general followed the recommendations of the Guidelines for the Management of Hypertension published in 2009 by the Japanese Society of Hypertension Committee (JSH 2009) (14) for BP above 220/120 mmHg. According to the record of the highest systolic and/or diastolic BPs during the first 72 hours after symptom onset, the patients were divided into three groups; control patients with normotension to mild hypertension (systolic BP <160 mmHg and diastolic BP <100 mmHg), patients with moderate hypertension (systolic BP 160 mmHg to 199 mmHg and/or diastolic BP 100 to 119 mmHg), and those with severe hypertension (systolic BP ≥200 mmHg and/or diastolic BP ≥120 mmHg).

Assessment of Brain Lesion

All patients underwent brain magnetic resonance imaging (MRI) and MR angiography on the first day of admission. Imaging was performed using a 1.5-T Siemens MRI (Model: MAGNETOM Avanto). Acute ischemic lesions were assessed by diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC). Ischemic lesions on DWI were classified into single lesions (corticosubcortical lesion, cortical lesion, subcortical lesion with a diameter ≥15 mm, or subcortical lesion with a diameter <15 mm), scattered lesions in one vascular territory (small [<15 mm] scattered lesions or confluent [≥15 mm] lesions with an additional lesion), and multiple lesions in multiple vascular territories (in the unilateral anterior circulation, posterior circulation, in bilateral anterior circulations, or anterior and posterior circulations). Stroke subtype classification was performed according to a previous report (15). Patients with DWI imaging findings of a corticosubcortical single lesion and those with multiple lesions both in the anterior and posterior circulation were classified as having cardiac embolism if they had atrial fibrillation and/or another cardioembolic source. Those with subcortical small lesion(s) (<15 mm) were classified as having small-vessel occlusion. Those with subcortical lesion(s) (<20 mm) were also classified as having small-vessel occlusion according to the recommendations in the report (15). Other patients with atrial fibrillation and/or another cardioembolic sources were classified as having cardiac embolism. Patients without a cardioembolic source were classified as having large-artery atherosclerosis. Patients with transient ischemic symptom with no visible lesion on DWI were excluded from this study.

Laboratory Procedures

Serum levels of total cholesterol, HDL-cholesterol, triglycerides, the acute phase reactant C-reactive protein (CRP), and albumin were measured using an automated spectrometer (Hitachi LABOSPECT-008). LDL-cholesterol level was calculated using the Friedewald formula (16). Measurements were made on admission (within 24 hours of stroke onset).

Underlying Chronic Conditions

We observed the clinical features of the enrolled patients including the following: past history of stroke, ischemic heart disease, chronic congestive heart failure, chronic kidney disease, diabetes mellitus, and hypertension under treatment with antihypertensive agents. Only chronic conditions were recorded for the patients. Operational definitions of each pre-existing chronic condition were established prior to data collection, including past history of stroke (evidence of chronic phase of stroke on magnetic resonance imaging), ischemic heart disease (evidence on electrocardiography and echocardiography), chronic congestive heart failure (left ventricular ejection fraction ≤40%), chronic kidney disease (estimated glomerular filtration rate calculated by the Modification of Diet in Renal Disease equation (17) with coefficients modified for Japanese patients (18), $194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times$ 0.739 if female) <60 ml · min^{-1} · 1.73 m^{-2}), diabetes mellitus (use of hypoglycemic agents and/or insulin), and hypertension (use of antihypertensive agents). As a consequence, out of the 53 elderly subjects with hypertension using antihypertensive agents before the stroke, 12 were prescribed an angiotensin II-receptor blocker (ARB) alone, 3 an angiotensin I-converting enzyme inhibitor (ACEI) alone, 13 a dihydropyridine calcium-channel blocker (CaB) alone, 2 a thiazide alone, and 23 two or more classes of antihypertensives out of the four classes.

Statistical Methods

Continuous variables were reported as mean with 95% confidence interval (CI) and compared by one-way ANOVA, with Tukey's test for post hoc comparisons. Serum triglycerides and C-reactive protein spread results were positively skewed, and were therefore log-transformed before statistical comparison. Results were then expressed in natural units for ease of interpretation. Discrete variables were reported as percentages and compared by χ^2 analysis. Logistic regression analysis was used to identify factors independently associated with severe hypertension after adjustment for confounding variables. Common pitfalls associated with multivariate regression were avoided as described by Cibcati et al. (19). The odds ratio (OR) for severe hypertension associated with various conditions was calculated by logistic regression analysis, adjusting for age, sex, and all associated variables selected according to their univariate analysis p value ($p<0.10$). Estimates for OR and corresponding two-sided 95% CI demonstrating statistical significance were derived from the regression model. Conditional logistic regression was used to control for potential confounding variables. Data were analyzed using SPSS (v. 16.0, Chicago, IL, USA). A probability of $p<0.05$ was taken as statistically significant.

Table 1. Characteristics of controls, moderate hypertensives, and severe hypertensives

	Controls n = 55	Moderate hypertensives n = 87	Severe hypertensives n = 20	p value
Clinical background				
Age (years)	83.8 (82.4-85.1) [72-95]	85.9$^{\#}$ (84.7-87.1) [71-97]	84.7 (82.8-86.5) [80-94]	0.064
Male: female	34: 21	43; 44	8: 12	0.174
Type of ischemic stroke (n)				
Large-artery atherosclerosis	32	52	12	
Small-vessel occlusion	10	11	4	
Cardiac embolism	13	12	4	
BMI (kg/m^2)	20.2 (18.9-21.4)	19.8 (18.6-21.0)	18.1 (13.1-23.0)	0.444
Systolic BP (mmHg)	139.5 (134.9-144.1)	178.2*** (175.6-180.8)	214.3***$^{+++}$ (208.1-220.4)	<0.001
Diastolic BP (mmHg)	79.8 (76.9-82.6)	95.2*** (92.3-98.0)	108.6***$^{+++}$ (101.3-115.8)	<0.001
Pulse pressure (mmHg)	59.7 (55.9-63.5)	83.0*** (79.8-86.2)	105.7***$^{+++}$ (98.0-113.4)	<0.001
Glasgow Coma Scale	14.2 (13.7-14.6)	12.6** (11.9-13.2)	11.7** (10.3-13.1)	<0.001
White blood cell count (x 10^9/L)	6.92 (6.33-7.52)	6.50 (6.04-6.95)	8.62**$^{++}$ (7.09-10.1)	0.001
Serum C-reactive protein (mg/L)	3.87 (2.56-5.86)	3.27 (2.46-4.35)	4.96 (2.55-9.67)	0.458
Serum albumin (g/L)	36.0 (34.9-37.2)	35.1 (34.1-36.1)	34.9 (32.1-37.7)	0.477
Underlying chronic conditions				
Past history of stroke (%)	36.4	24.4	35.0	0.247
Ischemic heart disease (%)	3.6	6.9	5.6	0.710
Congestive heart failure (%)	23.6	17.2	20.0	0.652
Chronic kidney disease (%)	5.5	8.0	20.0$^{\#}$	0.136
Diabetes mellitus (%)	12.7	11.5	15.0	0.908
Hypertension treatment (%)	21.8	41.4*	25.0	0.039
ARB alone (%)	5.5	10.3	0	0.226
ACEI alone (%)	1.8	1.1	5.0	0.520
CaB alone (%)	5.5	9.2	10.0	0.688
Thiazide alone (%)	1.8	2.3	0	0.792
≥Two antihypertensives (%)	7.3	19.5	10.0	0.107
ARB with/without others (%)	10.9	20.7	10.0	0.225
ACEI with/without others (%)	1.8	9.2	5.0	0.203
CAB with/without others (%)	12.7	21.8	20.0	0.394
Thiazide with/without others (%)	3.6	9.2	0	0.195

Results for continuous variables are expressed as mean (95% CI) [range] value and compared using one-way ANOVA with Tukey's *post hoc* analysis. Discrete variables are reported as percentages and compared with χ^2 analysis. BP: blood pressure, ARB: angiotensin II-receptor blocker, ACEI: angiotensin I-converting enzyme inhibitor, CaB: dihydropiridine calcium-channel blocker. $^{\#}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. controls. $^{+}p<0.10$, $^{++}p<0.05$, $^{+++}p<0.01$, $^{++++}p<0.001$ vs. moderate hypertensives.

RESULTS

Twenty patients with severe hypertension were compared with 87 patients with moderate hypertension and 55 controls (Table 1). Mean age tended to be higher in the moderate hypertension group than in the control group. In addition to the differences in BP, the score for Glasgow Coma Scale was significantly lower in the group with severe hypertension than in the control group, and blood white blood cell count was significantly higher in the severe hypertension group than in either of the other two groups. The rate of antihypertensive treatment prior to ischemic stroke was significantly higher in the moderate hypertension group than in the control group (Table 1).

Serum level of total and HDL-cholesterol and triglycerides were similar in all three groups (Table 2). The most notable finding, however, was the higher LDL-cholesterol level in the severe hypertension group than in the control group ($p = 0.013$). The LDL/HDL-cholesterol ratio was therefore significantly higher in the severe hypertension group than in the control group.

A series of multivariate logistic regression analyses were performed to identify whether differences in lipid profile were independent of the effects of age, sex, score for Glasgow Coma Scale, white blood cell count, or antihypertensive treatment prior to ischemic stroke

Table 2. Lipid parameters in controls, moderate hypertensives, and severe hypertensives

	Controls	Moderate hypertensives	Severe hypertensives	ANOVA p
Total cholesterol (mmol/l)	4.65 (4.45-4.85)	4.75 (4.51-5.00)	5.24#! (4.84-5.64)	0.074
LDL-cholesterol (mmol/l)	2.95 (2.77-3.13)	3.10 (2.91-3.29)	3.59*+ (3.23-3.94)	0.013
HDL-cholesterol (mmol/l)	1.20 (1.12-1.28)	1.19 (1.12-1.26)	1.15 (0.99-1.30)	0.808
LDL/HDL-cholesterol ratio	2.63 (2.37-2.89)	2.75 (2.53-2.97)	3.30*! (2.74-3.86)	0.046
Serum triglycerides (mmol/l)	1.00 (0.90-1.11)	0.90 (0.82-0.99)	0.99 (0.81-1.19)	0.349

Results for continuous variables are expressed as mean with 95% CI in parentheses and compared using one-way ANOVA with Tukey's *post hoc* analysis. Keys as in Table 1.

Table 3. Adjusted odds ratio for comparison of severe hypertensives with controls

Lipid parameter	1 SD of total sample	Adjusted odds ratio for 1 SD change	95%CI	*p* value
Total cholesterol (mmol/l)	1.012	5.69	1.97-16.5	0.001
LDL-cholesterol (mmol/l)	0.839	5.87	2.07-16.6	0.001
HDL-cholesterol (mmol/l)	0.320	1.02	0.53-1.96	0.954
LDL/HDL-cholesterol ratio	1.045	2.05	1.12-3.74	0.020
Log triglycerides (mmol/l)	0.189	0.77	0.43-1.76	0.701

The odds ratio refers to the change in likelihood of malignant hypertension for a 1 SD increase in predictor variable. Each parameter was entered into a separate logistic regression model with age, sex, Glasgow Coma Scale, white blood cell count and antihypertensive treatment prior to ischemic stroke.

Table 4. Adjusted odds ratio for comparison of severe hypertensives with moderate hypertensives

Lipid parameter	1 SD of total sample	Adjusted odds ratio for 1 SD change	95%CI	*p* value
Total cholesterol (mmol/l)	1.012	1.70	0.99-2.94	0.056
LDL-cholesterol (mmol/l)	0.839	1.87	1.06-3.31	0.031
HDL-cholesterol (mmol/l)	0.320	0.95	0.55-1.65	0.862
LDL/HDL-cholesterol ratio	1.045	1.50	0.93-2.40	0.094
Log triglycerides (mmol/l)	0.189	1.28	0.73-2.23	0.396

The odds ratio refers to the change in likelihood of malignant hypertension for a 1 SD increase in predictor variable. Each parameter was entered into a separate logistic regression model with age, sex, Glasgow Coma Scale, white blood cell count and antihypertensive treatment prior to ischemic stroke.

Table 5. Adjusted odds ratio for comparison of moderate hypertensives with controls

Lipid parameter	1 SD of total sample	Adjusted odds ratio for 1 SD change	95%CI	*p* value
Total cholesterol (mmol/l)	1.012	1.23	0.83-1.84	0.306
LDL-cholesterol (mmol/l)	0.839	1.39	0.93-2.08	0.113
HDL-cholesterol (mmol/l)	0.320	1.00	0.68-1.48	0.989
LDL/HDL-cholesterol ratio	1.045	1.21	0.80-1.81	0.368
Log triglycerides (mmol/l)	0.189	0.72	0.48-1.06	0.093

The odds ratio refers to the change in likelihood of malignant hypertension for a 1 SD increase in predictor variable. Each parameter was entered into a separate logistic regression model with age, sex, Glasgow Coma Scale, white blood cell count and antihypertensive treatment prior to ischemic stroke.

(Tables 3, 4 and 5). These analyses showed that while the differences in LDL-cholesterol level between the severe hypertension group and the other two groups remained (Table 3, 4), there were no differences in serum triglycerides level or measures of HDL-cholesterol. The difference in total cholesterol level between the severe hypertension group and the control group was also significant after adjustment for these confounding factors (Table 3). However, there was no difference in any of the lipid profile between the moderate hypertension group and control group (Table 5). The differences in levels of total cholesterol and LDL-cholesterol between the severe hypertension group and the control group remained even after adjustment for use of each antihypertensive treatment as a confounding factor instead of antihypertensive treatment prior to ischemic stroke, as an ARB alone or with another antihypertensives agents (LDL-cholesterol; OR: 6.11, 95% CI: 2.10-17.8, p=0.001, total cholesterol; 5.86, 2.00-17.2, p=0.001), ACEI alone or with other antihypertensives (LDL-cholesterol; 6.43, 2.20-18.8, p=0.001, total cholesterol; 5.88, 2.03-17.0, p=0.001), and CaB alone or with other antihypertensives (LDL-cholesterol; 5.54, 1.96-15.7, p=0.001, total cholesterol; 5.40, 1.87-15.5, p=0.002).

Conditional logistic regression analysis using that the same confounding factors revealed the differences in both total cholesterol (OR: 8.14, 95% CI: 1.77-37.4, $p = 0.007$) and LDL-cholesterol (OR: 9.33, 95% CI: 1.77-49.2, $p = 0.009$) between the severe hypertension group and the control group remained independent in the subgroup with large-artery atherosclerosis (n=96).

DISCUSSION

In this study, we attempted to compare patients presenting with severe hypertension to normotensive/mild hypertensive control patients and patients with moderate hypertension, in the acute phase of ischemic stroke. It is not possible to be certain that subclinical vascular diseases disturbing lipid metabolism was not present in any of the study participants. However, the use of pre-defined criteria for the definition of vascular disease applied to all three study groups minimizes the likelihood of confounding by vascular disease. Indeed, the distributions of patients with diabetes mellitus, or ischemic heart disease were similar among the three groups. Although chronic kidney disease may cause dyslipidemia characterized by a decrease in HDL-cholesterol level (20), the distribution of patients with chronic kidney disease was also similar among the three groups.

Another potential confounder may be drug therapy. Several drugs used for antihypertensive therapy are known to modify lipoprotein metabolism. Thiazides at a high dosage can increase serum LDL-cholesterol, triglycerides, and the total cholesterol/ HDL-cholesterol ratio, while HDL-cholesterol is largely unchanged (21). ARBs are known to decrease serum levels of total cholesterol, LDL-cholesterol, triglycerides, and the total cholesterol/ HDL-cholesterol ratio, while HDL-cholesterol is largely unchanged (22). ACEI may slightly decrease serum triglycerides (21). Some CaB can also increase serum HDL-cholesterol level (23). In the present study, 53 out of the 162 patients (33%) had been treated with an antihypertensive agent prior to acute ischemic stroke, including an ARB alone, ACEI alone, CaB alone, thiazide alone, or two or more classes of antihypertensives agents out of the four classes. The rate of antihypertensive treatment prior to ischemic stroke was significantly higher in the moderate hypertension group than in the control group. However, there was no significant difference in rate of single or combination use of particular antihypertensives agents between the severe hypertensive group and either the control group or moderate hypertensive group. Moreover, the differences in levels of total cholesterol and LDL-cholesterol between the severe hypertension group and the control group remained even after adjustment for the use of each antihypertensive treatment as a confounding factor rather than antihypertensive

treatment prior to ischemic stroke. These results indicate that effect(s) of any antihypertensive treatment prior to ischemic stroke on dyslipidemia in the severe hypertensive group is minimal.

Another potential confounder may be the acute-phase response. The acute-phase response may be responsible for reductions in both LDL- and HDL-cholesterol levels and an increase in serum triglyceride level (24). Acute ischemic stroke is not always associated with an acute-phase response. Serum levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were reported to remain stable following acute ischemic stroke, consistent with the absence of acute phase response or nutritional deficiency (25). In the present study, however, some of the elderly patients with acute ischemic stroke may have been complicated by infectious diseases, since white blood cell count was increased especially in the with severe hypertension group. Patients with severe infections may also have an associated acute-phase response, with those circulating LDL-cholesterol levels closely mirroring plasma CRP levels (26). It is difficult to estimate the extent to which the results in the severe hypertension group may have been affected by an acute-phase response, but the increased LDL-cholesterol level seen in the severe hypertension group was in the opposite direction to the change in LDL-cholesterol in the acute phase response caused by infection.

In the present study, serum samples were not always obtained from patients after overnight fasting. However, serum triglycerides levels in all subjects were below 4.52 mmol/L (data not shown), suggesting effect(s) of non-fasting state for application of Friedewald formula for LDL-cholesterol may be minimal.

This study demonstrates that severe hypertension in elderly patients with acute ischemic stroke might be associated with an abnormal lipid profile, characterized by high LDL-cholesterol and total cholesterol levels. The precise mechanism(s) of the association is not clear. However, hypercholesterolemia may induce cerebrovascular supersensitivity to catecholamine in an animal model (27). More work is needed to better define dyslipidemia in severe post-stroke hypertension, which may be at least in part responsible for the poor prognosis of these patients (6-9).

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症例報告

化膿性膝関節炎を感染巣とし劇症型A群β溶連菌感染症を呈した1例

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【要旨】 劇症型A群β溶連菌感染症（Streptococcal Toxic Shock Syndrome，以下STSS）は急速な経過をたどり，致命率の高い重症感染症である。今回，38歳の男性が化膿性膝関節炎および急激に進行したショック状態で当院に紹介搬入された。膝関節が感染源のSTSSと診断し，全身管理および外科的処置にて救命した。STSSはおもに軟部組織感染で発症するといわれているが，本症例は化膿性膝関節炎で発症したと考えられた。

索引用語：劇症型A群β溶連菌感染症，化膿性膝関節炎，敗血症性ショック

はじめに

劇症型A群β溶連菌感染症（Streptococcal Toxic Shock Syndrome：以下STSS）は，症状の進行がきわめて急激かつ劇的であり，いったん発病すると数十時間以内に軟部組織壊死，急性腎不全，急性呼吸窮迫症候群，播種性血管内凝固症候群など多臓器不全を引き起こしショック状態から死に至る恐ろしい疾患である[1,2]。今回，本症と診断し，救命しえた症例を経験したので報告する。

症　例

患　者：38歳，男性
主　訴：左膝関節痛

A Case of Streptococcal Toxic Shock Syndrome Induced by Septic Arthritis of the Knee Joint
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既往歴：尿糖陽性を指摘されたことあり

現病歴：左膝関節痛を主訴とし近医を受診。関節穿刺で膿汁排泄を認め，排液の培養にてグラム陽性球菌が陽性であったため，化膿性膝関節炎の診断で受診2日後同院に入院した。抗菌薬（塩酸セフォチアム）が投与されたが，入院当日夜より急激な血圧低下からショック状態となり，敗血症性ショックの疑いで当院当科に紹介された。当院に来院するまでの経過中に発熱は認めなかった。

入院時現症：意識清明，皮膚は湿潤，冷汗著明，血圧60/mmHg（塩酸ドパミン15μg/kg/min投与下），脈拍140/分，整，呼吸30/分，体温36.0℃，口腔咽頭に異常所見なし，呼吸音および心音正常，腹部の理学的所見に異常なし。左膝関節は発赤，腫脹，熱感あり。紹介医によって左膝関節に留置されたドレーンからは黄白色の膿性排液を認めた。膝関節以外の左下肢には明らかな創傷はなく，趾間に白癬も認めなかった。

入院時血液生化学検査（表1）：白血球は4,540/μlで，近医での2日前の採血結果（19,000/μl）より急激な減少を認めた。CRPの著明な上昇と腎機能障害，肝機能障害，凝固機能異常を認めた。HbA1cは5.6％で正常範囲内であった。来院時の

表1　入院時血液生化学検査所見

RBC	4.36 × 10^6 /μl	Na	139 mEq/l
Hb	14.8 g/dl	K	3.4 mEq/l
Ht	42.8 %	Cl	101 mEq/l
WBC	4540 /μl	BUN	45 mg/dl
Neutro	92.6 %	Cre.	4.68 mg/dl
PLTS	14.6 × 10^4 /μl	T-bil	2.0 mg/dl
CRP	36.24 mg/dl	D-bil	1.3 mg/dl
		LDH	301 U/l
PT（INR）	1.32	AST	77 U/l
PT（%）	56.0 %	ALT	64 U/l
APTT	62.4 sec	CK	522 U/l
Fibrinogen	703 mg/dl	Glu	262 mg/dl
FDP	64.8 μg/ml	HbA1c	5.6 %
D-dimer	48.32 μg/ml	endotoxin	3.5 以下
AT Ⅲ	52.1 %	β -D glucan	6 以下

WBCの低下とCRPの著明な上昇および腎機能障害，肝機能障害，凝固機能異常を認める。

APACHE- Ⅱ scoreは16点で，院内予測死亡率は23.5％であった。また，感染源検索のため胸腹部のX線およびCT検査，尿検査を施行したが異常所見を認めなかった。紹介医より膝関節穿刺液よりグラム陽性球菌が培養中と連絡があったため，ブドウ球菌感染によるToxic shock syndrome（以下TSS）もしくは溶血性連鎖球菌感染によるSTSSを疑い治療を開始した。

入院後経過（図1）：全身管理目的にICUに入室させた。治療はSurviving sepsis campaign guidelinesに基づき行った。抗菌薬は，前医で第3世代セフェムを投与されていたものの効果がなかったため，起因菌が判明するまで広域抗菌薬としてイミペネム／シラスタチンナトリウム2g/day，クリンダマイシン2.4g/dayを投与し，γ-グロブリン5g/dayを投与した。抗DIC療法としてメシル酸ガベキサート1500mg/day，ウリナスタチン20万単位/day，AT-Ⅲ製剤1500単位/dayを投与した。またPT値が30％以上に保つように，随時新鮮凍結血漿を投与した。敗血症性ショックによる腎不全があり，循環動態が不安定なことと体液性メディエータ除去目的に持続的血液濾過透析（以下CHDF）を施行した。この症例はグラム陽性菌感染であることが判明していたが，グラム陽性菌もToll様受容体を介して内因性大麻を誘導し，PMXカラムが内因性大麻を吸着・除去することより，ポリミキシンB固定化ファイバーを用いた直接血液灌流療法（以下PMX-DHP）を入院当日に1回施行した。PMX-DHP施行後は白血球が上昇し，尿量が確保されるようになったが，血圧の上昇や頻脈の改善はなかった。サイトカイン値は，入院した日が大型連休中であったこともあり，今回の症例では測定しなかった。また，感染源のコントロールとして入院当日より1日1回，2Lの生理食塩水で膝関節間欠的洗浄を行い，関節内に塩酸アミカシンを投与した。大腿後面から膝関節にかけて発赤を認めたが，壊死性筋膜炎には陥っていなかった。酸素化は経時的に悪化し，胸部X-Pで両側肺野のびまん性浸潤影を認め，PaO_2/FiO_2比が60と著明な低下を示したため，急性呼吸促迫症候群（以下ARDS）と診断し，第2病日未明より人工呼吸管理を開始した。自発呼吸がしっかりしていたことと，血行動態が不安定で鎮静薬の使用量を制限したため，同期的間歇的強制換気（従圧式）＋圧支持換気，呼気終末陽圧10cmH_2Oで管理を開始し，1回換気量は6ml/kg程度になるようにした。同時にシベレスタットナトリウム400mg/dayの投与を開始した。気管チューブからは大量の血性泡沫痰を認めた。血小板が30,000/μlまで低下したため，血小板輸血（30単位）を第2病日より3日間施行した。血圧は塩酸ドパミン＋塩酸ドブタミン＋ノルアドレナリンを使用したが，第2病日までは収縮期血圧が70mmHg台で推

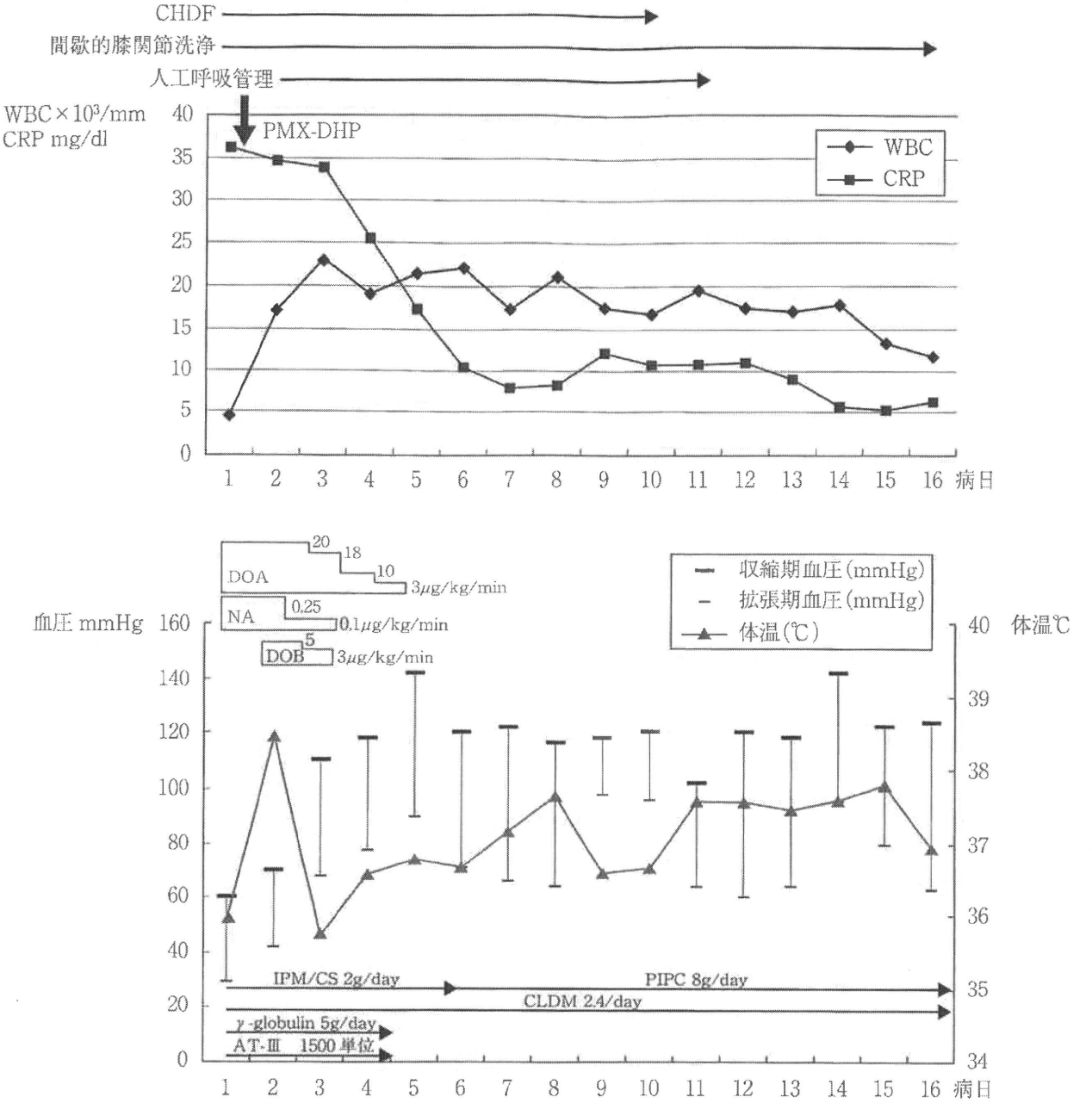

図1　入院後治療経過

入院当日よりCHDF開始。抗菌薬はIPM/CSとCLDMを投与。感染源対策として間歇的膝関節洗浄を施行。

移した。第3病日より循環動態が安定してカテコラミン減量が可能となり，第5病日で中止となった。当院での膝関節液の培養でA群β溶連菌が検出されたため，第6病日より抗生剤をピペラシリンナトリウム（以下PIPC）に変更した。なお，来院時に血液培養も試行したが，病原菌は陰性であった。第10病日にはCHDFを離脱，第11病日には抜管可能となった。第16病日には全身状態が安定したため，化膿性膝関節炎の根本的治療のため整形外科に転科となった。転科後，第22病日に膝関節のMRIを撮影したところ，T2脂肪抑制像で膝関節周囲に高信号を認めた。炎症性変化は大腿骨周囲に沿って上方に広がり，コンパートメント症候群様の変化を呈していた（図2）。また第23病日のCTでは大腿骨遠位端周囲に膿瘍を有し，一部airを伴っており，周囲の脂肪織の肥厚もみられた（図3）。第45病日のGaシンチでは左膝のみに集積を認めた（図4）。整形外科転科後も膝関節洗浄および抗生剤投与

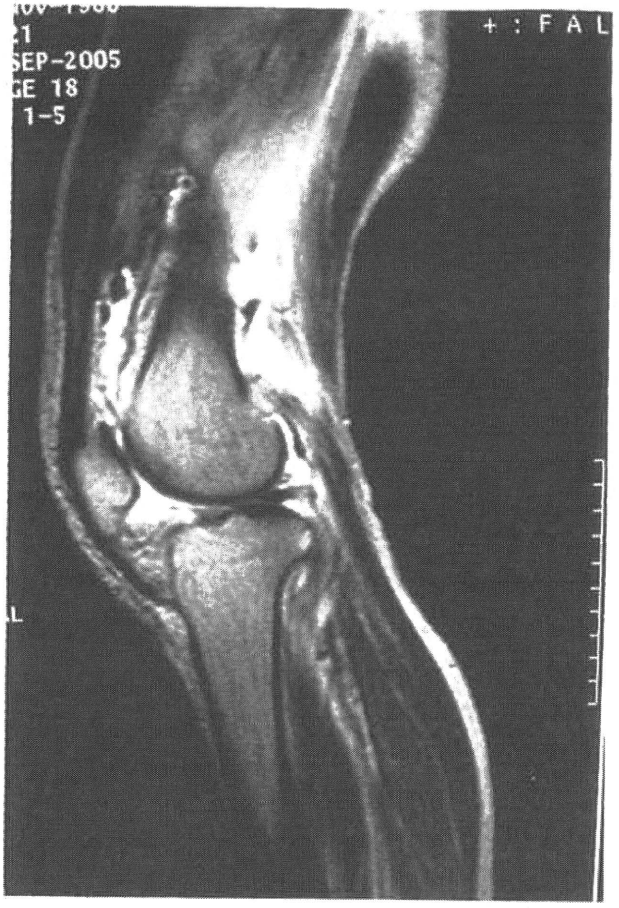

図2　全身状態改善後第22病日の左膝MRI
T2脂肪抑制像で膝関節周囲に高信号。

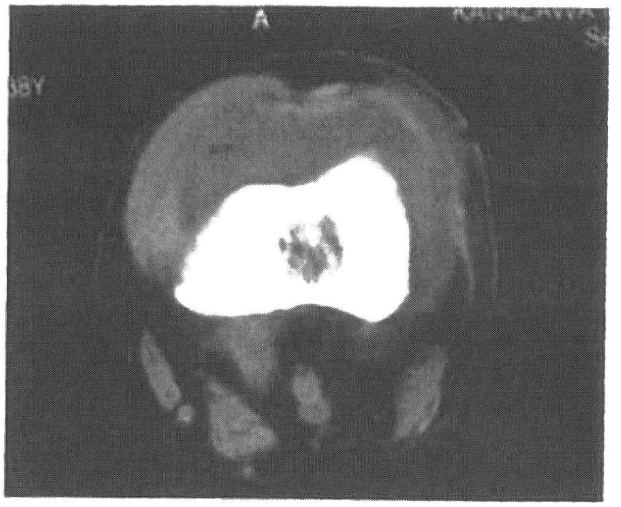

図3　全身状態改善後第23病日の左膝CT
大腿骨遠位端周囲に膿瘍を有し，一部airをみとめる。また周囲の脂肪織の肥厚あり。

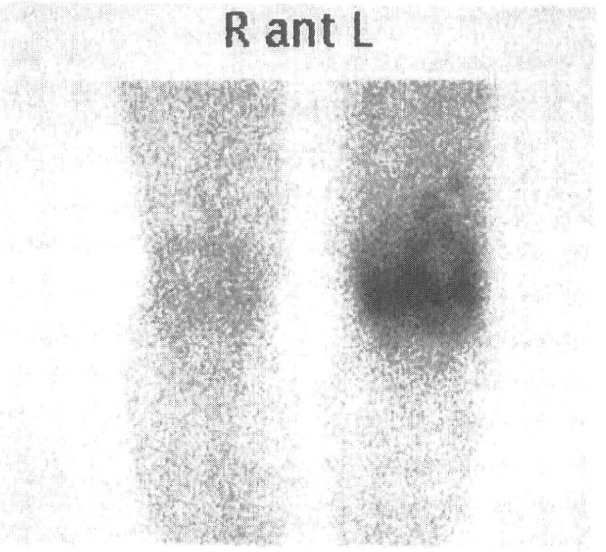

図4　第45病日のGaシンチ
左膝のみに集積あり。

(PIPC) を継続したが炎症反応が鎮静化せず，また高度の関節拘縮をきたしたため，第61病日目に関節鏡下滑膜切除術および関節受動術が施行された。その後炎症反応は消退し，術後はリハビリテーションの試行で関節可動域も改善し，第117病日に杖歩行で社会復帰した。膝関節液の培養は入院中に5回検査したが，入院時にA群β溶連菌が陽性になった以外はいずれも病原菌なしで，幸いにして菌交代現象は起こらなかった。

考　察

本症例は膝関節穿刺液よりA群β溶連菌が検出され，低血圧，腎障害，凝固異常の重症臨床所見を呈し，CDC診断基準を満たした[3]ためSTSSと診断した。STSSの治療は早期診断，早期治療を原則として，早期の外科的治療，全身管理とペニシリン系抗生剤投与，γ-グロブリン投与とされている。抗生剤は，A群溶連菌はβラクタム系薬剤には耐性を示しておらず，良好な感受性を示すためペニシリン系が第1選択となる。また，外毒素の産生を抑制し細胞内移行性の高いクリンダマイシンも推奨されている[3]。しかし，ペニシリン系抗菌薬およびクリンダマイシンの優劣や併用効果についての臨床的検証は行われていない。また，γ-グロブリンは外毒素の中和作用をもつと報告されており，多くの論文では使用を推奨しているが，グロブリン製剤の使用の有無にかかわらず死亡率に差はなかったという後

ろ向き研究もある[6]。また，敗血症性膝関節炎の治療法に関してはルーチンで関節鏡下のデブリードマンが推奨されており，さらに滑膜増殖を認める場合は滑膜切除術を考慮すべきであるといわれている[7]。

A群β溶連菌感染による敗血症性膝関節炎の報告は少なく，われわれが医中誌で検索しえた限りでは本例を含め2例のみであった[4]。いずれも男性で，膝関節痛を初発症状として発症した。自験例では，当初TSSの可能性も考え抗菌薬は広域スペクトラムのIPM/CSを投与したが，培養結果が判明した第6病日目からはPIPCに変更した。外科治療としては，転院時はショック状態を呈しており，全身麻酔下の手術は不可能であったため，まずは感染巣のコントロールとして膝関節の間歇的洗浄を施行した。その後，関節拘縮をきたしたため関節鏡下滑膜切除術および関節受動術となった。手術所見では関節内に膿の貯留はなく，滑膜の増生が著明であった。他の報告例では，化膿性膝関節炎に対して関節鏡下滑膜切除術施行後3日目にSTSSの状態に陥り，全身管理後に2度の外科的手術施行後，社会復帰している[4]。報告例では，救命しかつ患肢を温存できたのは，関節包がバリアとなり，細菌の増殖および外毒素の影響が関節内にとどまったためと考察されていて，本症例もこの考察を支持する結果となったが，2例のみの結果であるため，今後さらなる検討が必要と考えられる。本症例で，外科的手術（滑膜切除術）を行うタイミングに関しては，整形外科転科後，第61病日目に行われており，やや消極的であったといわざるを得ない。しかし，重篤化した症例での外科的処置を行うタイミングは難しい。本症例ではCHDFを含めた早期からの集中治療が功を奏し，病原巣のコントロールも加えて行ったことが救命につながったのではないかと考えている。

本邦では1992年にSTSSの典型例が報告され，以後国立感染症研究所感染症情報センターには，1999年から2006年までにのべ473例の患者が報告されている[8]。死亡率は約45%というきわめて致死率の高い感染症であるが，侵入経路が不明であったり，先行する感染巣を認めないことがあり，診断が難しい[9]。しかし，的確な治療開始の遅れは致死的な状態を引き起こす可能性が高いため，いかなる場合でもSTSSの可能性を当初より念頭におき，治療にあたる必要があると考えられる。

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Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections

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Abstract

Streptococcus dysgalactiae ssp. *equisimilis* (SDSE) is increasingly being identified as a pathogen responsible for invasive and non-invasive infections. We compared the clinical features of invasive SDSE infections with those of invasive infections caused by *Streptococcus pyogenes* (group A streptococcus (GAS)) and *Streptococcus agalactiae* (group B streptococcus (GBS)). Active surveillance for invasive SDSE, GAS and GBS was maintained over 1 year at 142 medical institutions throughout Japan. Clinical information was collected together with isolates, which were characterized microbiologically. Two hundred and thirty-one invasive SDSE infections were identified, 97 other patients had infections with GAS, and 151 had infections with GBS. The median age of the SDSE patients was 75 years; 51% were male and 79% had underlying diseases. Forty-two SDSE patients (19%) presented to the emergency department. Among the 150 patients (65%) for whom follow-up was completed, 19 (13%) died and eight (5%) had post-infective sequelae (poor outcome). Insufficient white blood cell responses (<5000 cells/μL) and thrombocytopenia on admission each suggested significantly higher risk of poor outcome (ORs 3.6 and 4.5, respectively). Of 229 isolates, 55 (24%) showed an stG6792 *emm* type, which was significantly associated with poor outcome (OR 2.4). Clinical manifestations of invasive SDSE infections were distinct from those of invasive GBS infections. Primary-care doctors should consider invasive SDSE infections when treating elderly patients.

Keywords: Invasive infections, non-invasive infections, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* ssp. *equisimilis*

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Introduction

Invasive infections caused by β-haemolytic streptococci apart from Lancefield groups A and B, as well as by *Streptococcus pyogenes* (group A streptococcus (GAS)) and *Streptococcus agalactiae* (group B streptococcus (GBS)), are reported increasingly worldwide [1,2]. The other streptococci include groups C, G, F and L; group G is notable because these streptococci can cause bacteraemia [3,4]. According to previous investigations [5], this group includes *Streptococcus dysgalactiae* ssp. *equisimilis* (SDSE), the *Streptococcus anginosus* group, and *Streptococcus canis*.

Recently, SDSE isolates possessing group G antigen have been recovered increasingly from severe invasive streptococcal infections [6]. Brandt *et al.* [7] characterized blood culture isolates of SDSE possessing Lancefield group A antigen. Infection with this pathogen (11 strains) was also sometimes found to lead to streptococcal toxic shock syndrome [8]. We have just completed whole genome analyses of two original isolates (GGS_124 (GenBank accession number

AP010935) and RE378) of SDSE, demonstrating a rate of genome overlap between this subspecies and GAS of 61–63%, whereas the overlap between the subspecies and GBS genomes was 15% (T. Akiyama, K. Ubukata & T. Kirikae, unpublished data).

However, active nationwide surveillance with a collection of large numbers of strains remains to be instituted, as for many years SDSE was considered to be non-pathogenic. We therefore collected isolates of this microorganism as well as of GAS and GBS, with accompanying detailed clinical information, from 142 medical institutions. Our aim was to compare clinical aspects of invasive diseases caused by SDSE with those caused by GAS or GBS in Japan.

Materials and Methods

Surveillance

Active laboratory-based surveillance for invasive SDSE, GAS or GBS infections was organized by the Laboratory of Molecular Epidemiology for Infectious Agents at the Graduate School of Infection Control Sciences, Kitasoto, Japan. SDSE included some isolates of the Lancefield C or A groups. We excluded *S. anginosus* group isolates that showed group C, G or F antigen in the process of isolate identification.

Surveillance was conducted for 1 year (1 August 2006 to 31 July 2007) in 142 medical institutions participating in the Invasive Streptococcal Disease Working Group established at the 19th Annual Meeting of the Japanese Society for Clinical Microbiology. Invasive streptococcal diseases were defined as conditions with isolation of organisms from a normally sterile site (i.e. blood, cerebrospinal fluid, joint fluid, ascites, or pleural effusion) [1,9,10]. Isolates were first identified as streptococci at local hospital laboratories, using standard commercially available latex agglutination assays. Detailed standardized categories of information regarding clinical features (e.g. hospital departments of initial presentation and underlying conditions) and laboratory findings (e.g. white blood cell (WBC) count, platelet count and C-reactive protein (CRP) serum concentration) were obtained from medical charts for all subjects enrolled. Clinical syndromes were assigned on the basis of physicians' diagnoses recorded in the medical charts. Poor outcomes were defined as either death from invasive infections within 3 weeks of onset or invasive disease-associated sequelae following the streptococcal infection (e.g. worsened paralysis or bedridden status). All isolates were sent to the Laboratory of Molecular Epidemiology for Infectious Agents to determine additional characteristics, including Lancefield group, species, M protein gene (*emm*) or capsular type, and antibiotic susceptibility.

Laboratory methods

Isolates were characterized with standard biochemical and enzymatic tests, and were identified as previously described [11]. The *emm* types of SDSE or GAS isolates [12] and the capsular types of GBS [13] isolates were determined as previously reported. All *emm* typing was based on the CDC database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/). In addition, we quantified the susceptibility of streptococci to 14 antibiotics, including oral and parenteral antibiotics, by agar plate dilution methods using blood agar, as previously described [10]. Depending on the MICs, the presence of streptococcal genes associated with resistance to antimicrobials (e.g. *mef*(A), *erm*(A), or *erm*(B)) was determined. To assess the similarity of isolates, profiling using pulsed-field gel electrophoresis (PFGE) following DNA treatment with the restriction enzyme *Sma*I was also performed [10].

Statistical analysis

Microsoft Excel was used for data analysis. Patient or pathogen characteristics, clinical features and outcomes were compared between paired groups of isolates (SDSE and GAS, SDSE and GBS, or GAS and GBS), using the chi-squared test. To identify clinical laboratory findings associated with fatal outcome, ORs with 95% CIs and p-values according to the chi-squared test were calculated.

Results

We identified 231 patients with invasive infection caused by SDSE in the records from 142 medical institutions during the 1-year study period, during which time 97 GAS and 151 GBS cases were also collected. All isolates of SDSE, GAS or GBS were referred by the hospital laboratories for further microbiological characterization.

As shown in Fig. 1, the age distribution differed significantly between patients with invasive SDSE and those with GBS infection. All patients (n = 231) with invasive SDSE infection were adults, whereas GBS infected some patients 4 months old or younger in addition to adults, especially the elderly. We therefore chose to compare clinical aspects of invasive SDSE diseases with those caused by GAS (n = 82) or GBS (n = 123) in the adult population (≥15 years old).

Isolates (n = 12) of the *S. anginosus* group were excluded from the current surveillance. Lancefield groups G, C and A,

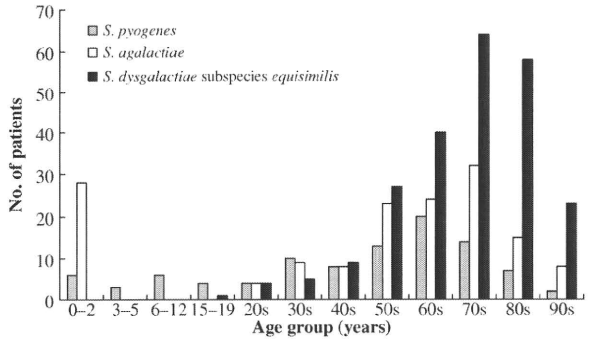

FIG. 1. Number of patients with invasive infections caused by *Streptococcus dysgalactiae* ssp. *equisimilis* (n = 231), *Streptococcus pyogenes* (n = 97), and *Streptococcus agalactiae* (n = 151), shown by age group.

respectively, accounted for 216, 12 and three SDSE isolates. All GAS or GBS isolates were *S. pyogenes* or *S. agalactiae*, respectively.

Patient characteristics and clinical presentations according to the streptococcal group (SDSE, GAS, or GBS)

Patient characteristics, clinical presentations and disease outcomes for all invasive SDSE, GAS and GBS infections are shown in Table 1. The median age of patients with SDSE diseases was 75 years (range, 19–103 years), with the subjects with SDSE infections being significantly older than those with GAS or GBS infections (p <0.01 for each). In our surveillance, all cases of SDSE, GAS and GBS diseases were community-acquired. Forty-two patients (19%) with SDSE infections presented to the hospital emergency department, a fraction similar to those of the subjects presenting to the emergency department with infections involving the other two groups.

Underlying diseases were present in 79% of patients with invasive SDSE illnesses; underlying medical conditions in subjects with GAS infections were significantly less frequent than

TABLE 1. Demographic characteristics, underlying conditions and clinical syndromes in patients with invasive infection caused by *Streptococcus dysgalactiae* ssp. *equisimilis* (n = 231), *Streptococcus pyogenes* (n = 97), or *Streptococcus agalactiae* (n = 151)

Characteristic	No. (%) of patients			p-Value		
	S. dysgalactiae ssp. *equisimilis* (A)	*S. pyogenes* (B)	*S. agalactiae* (C)	A vs. B	A vs. C	B vs. C
Demographic characteristic						
Age (years)						
<15	0 (0.0)	15 (15.5)	28 (18.5)	<0.01	<0.01	0.53
15–39	10 (4.3)	18 (22.0)	13 (10.6)	<0.01	0.02	0.03
40–64	50 (21.6)	32 (39.0)	39 (31.7)	<0.01	0.04	0.28
≥65	171 (74.0)	32 (39.0)	71 (57.7)	<0.01	<0.01	<0.01
Gender[a,b]						
Male	117 (51.3)	40 (49.4)	55 (44.7)	0.77	0.24	0.51
Department where presented[a]						
Emergency	42 (19.3)	22 (26.8)	22 (18.0)	0.15	0.78	0.13
Internal medicine	112 (51.4)	36 (43.9)	73 (59.8)	0.25	0.13	0.03
Surgery	64 (29.4)	24 (29.3)	27 (22.1)	0.99	0.15	0.25
Poor outcome[a]						
Death	19 (12.7)	12 (16.7)	8 (10.8)	0.42	0.69	0.30
Death or sequelae	27 (18.0)	19 (26.4)	11 (14.9)	0.15	0.56	0.08
Underlying disease[a]						
None	48 (21.2)	31 (39.7)	16 (11.8)	<0.01	0.07	<0.01
Diabetes mellitus	36 (15.9)	12 (15.4)	22 (16.2)	0.64	0.92	0.72
Malignant disease	35 (15.5)	13 (16.7)	33 (24.3)	0.39	0.02	0.41
Stroke	28 (12.4)	0 (0.0)	5 (3.7)	<0.01	<0.01	0.27
Heart disease	18 (8.0)	4 (5.1)	10 (7.4)	0.80	0.85	0.95
Pulmonary disease	2 (0.9)	0 (0.0)	0 (0.0)	0.93	0.72	–
Liver dysfunction	11 (4.9)	2 (2.6)	17 (12.5)	0.77	<0.01	0.03
Renal dysfunction	13 (5.8)	4 (5.1)	9 (6.6)	0.84	0.72	0.89
Artherosclerotic cardiovascular disease	8 (3.5)	1 (1.3)	11 (8.1)	0.66	0.05	0.12
Autoimmune disease	4 (1.8)	1 (1.3)	5 (3.7)	0.69	0.42	0.68
Other	23 (10.2)	10 (12.8)	8 (5.9)			
Clinical syndrome						
Sepsis without focus	98 (42.4)	27 (32.9)	77 (62.6)	0.06	<0.01	<.01
Cellulitis	52 (22.5)	23 (28.0)	12 (9.8)	0.43	<0.01	<.01
Septic arthritis	23 (10.0)	3 (3.7)	4 (3.3)	0.06	0.02	0.78
Pneumonia	12 (5.2)	6 (7.3)	8 (6.5)	0.74	0.64	0.87
Necrotizing fasciitis	9 (3.9)	5 (6.1)	1 (0.8)	0.67	0.18	0.08
Meningitis	5 (2.2)	3 (3.7)	3 (2.4)	0.79	0.82	0.97
Infectious endocarditis	4 (1.7)	0 (0.0)	4 (3.3)	0.51	0.60	0.24
Streptococcal toxic shock syndorome	2 (0.9)	3 (3.7)	0 (0.0)	0.25	0.76	0.13
Abscess (excluding skin)	2 (0.9)	8 (9.8)	3 (2.4)	<0.01	0.48	0.06
Osteomyelitis	2 (0.9)	0 (0.0)	1 (0.8)	0.94	0.59	0.85
Other	22 (9.5)[c]	4 (4.9)	10 (8.1)			

[a]Patients with unknown data were excluded.
[b]Gender, department where presented, poor outcome, underlying disease and clinical syndrome in the adult population were compared between paired groups (*S. dysgalactiae* ssp. *equisimilis* (SDSE) and group A streptococcus (GAS), SDSE and group B streptococcus (GBS), or GAS and GBS).
[c]Others included urosepsis (n = 13), septic spondylitis (n = 4), endophthalmitis (n = 2), peritonitis (n = 2), and biliary tract infection (n = 1).

in patients with SDSE or GBS infections (p <0.01 for each). Among patients with SDSE infection, stroke was significantly more frequent than in patients with GAS or GBS infection (p <0.01 for each), whereas in patients with GBS infection, liver dysfunction was significantly more prevalent than in those with SDSE or GAS infection (p <0.01 and p = 0.03, respectively). With regard to clinical syndromes, sepsis without focus was significantly more frequent among GBS-infected than among SDSE-infected or GAS-infected patients (p <0.01 for each), whereas cellulitis was significantly less frequent among GBS-infected patients than among SDSE-infected or GAS-infected patients (p <0.01 for each). Subjects infected with SDSE presented more often with septic arthritis than those infected with GBS (p = 0.02), whereas patients with GAS infections more often had abscesses involving sites deeper than the skin than did patients with SDSE infection (p <0.01).

Disease outcomes according to the streptococcal group (SDSE, GAS, or GBS)

Among 150 patients (65%) with invasive SDSE diseases for whom follow-up investigation was complete, 19 (13%) died, and eight (5%) had post-infection sequelae. No significant difference in frequency of poor outcome was evident among the three groups. The median time from admission to death was 3 days in subjects who died of SDSE infections, as compared with 1 and 7 days, respectively, for fatalities caused by GAS and GBS. Clinical laboratory data obtained at admission (WBC and platelet counts and CRP serum concentrations) for the three groups are shown in Table 2. In subjects with SDSE infection, a poor WBC response (<5000 cells/μL) and thrombocytopenia were associated with a significantly higher risk of poor outcome, with respective ORs of 3.6 (95% CI 1.2–11.5; p = 0.04) and 4.5 (95% CI 1.6–12.2; p <0.01). These two findings were also significantly related to poor outcomes in patients with GAS infections, but not in adults with GBS infections. There was no association of CRP level with poor outcome in the three groups.

emm typing for the isolates from adults

emm typing was carried out for 231 SDSE isolates and for 82 of the 97 *S. pyogenes* isolates; capsular typing was performed for 123 of the 151 *S. agalactiae* isolates. The incidences of *emm* types among SDSE isolates are shown in Table 3. Interestingly, *emm* type stG6792 in SDSE isolates, which was confirmed most frequently (n = 55, 24%), was significantly associated with poor outcome of invasive SDSE diseases in comparison with other SDSE *emm* types, with an OR of 2.4 (95% CI 1.0–5.9; p = 0.04). Within the stG6792 type, most isolates showed *emm* type stG6792.3 (n = 54), whereas PFGE of the isolates of the stG6792.3 type revealed similar profiles. On the other hand, among GAS isolates, *emm* type 1 was found most frequently (n = 27, 33%). This type was also significantly related to poor outcome of invasive infections, with an OR of 3.4 (95% CI 1.1–10.5; p 0.02). Other *emm* types frequently found in GAS infections were

TABLE 2. Clinical laboratory findings associated with fatal outcome of invasive infections caused by *Streptococcus dysgalactiae* ssp. *equisimilis*, *Streptococcus pyogenes*, or *Streptococcus agalactiae*

	Median or percentage (25/75th percentiles) and (no./total)			
	Poor outcome[a]	Without poor outcome[a]	Analytical data (OR (95% CI))	p-Value
S. dysgalactiae ssp. *equisimilis*				
WBC (/μL)	11 400 (3908–15 200) (22/27)	12 600 (7850–16 050) (107/123)	3.6 (1.2–11.5)	0.04
<5000/μL	27.3% (6/22)	9.3% (10/107)		
PLT (10^4/μL)	11.1 (7.3–15.2) (21/27)	18.8 (12.3–24.5) (100/123)	4.5 (1.6–12.2)	<0.01
<13.0 × 10^4/μL	66.7% (14/21)	13.0% (31/100)		
C-reactive protein (mg/dL)	18.7 (12.3–26.8) (22/27)	4.9 (1.6–21.1) (103/123)	0.2 (0.03–1.8)	0.23
<1 mg/dL	4.5% (1/22)	17.5% (18/103)		
S. pyogenes				
WBC (/μL)	7200 (3800–19 075) (18/19)	10 300 (8000–15 015) (46/53)	4.2 (1.2–15.6)	0.04
<5000/μL	38.9% (7/18)	13.0% (6/46)		
PLT (10^4/μL)	10.1 (7.4–16.5) (18/19)	19.4 (15.6–28.0) (46/53)	7.5 (2.2–25.8)	<0.01
<13.0 × 10^4/μL	61.1% (11/18)	17.4% (8/46)		
C-reactive protein (mg/dL)	22.1 (12.7–28.0) (17/19)	15.6 (5.4–24.6) (45/53)	0.6 (0.06–6.5)	0.89
<1 mg/dL	5.9% (1/17)	8.9% (4/45)		
S. agalactiae				
WBC (/μL)	14 350 (9200–17 225) (10/11)	11 600 (7800–15 150) (47/63)	1.2 (0.1–12.6)	0.64
<5000/μL	10.0% (1/10)	8.5% (4/47)		
PLT (10^4/μL)	10.0 (7.7–18.9) (9/11)	17.9 (12.2–24.3) (46/63)	3.2 (0.7–14.1)	0.23
<13.0 × 10^4/μL	55.6% (5/9)	28.3% (13/46)		
C-reactive protein (mg/dL)	6.7 (1.6–10.0) (9/11)	8.3 (1.1–19.0) (46/63)	1.2 (0.2–6.9)	0.78
<1 mg/dL	22.2% (2/9)	19.6% (9/46)		

WBC, white blood cell count; PLT, platelet count.
[a]Patients with unknown data were excluded.

TABLE 3. Types of *emm* for 231 cases of invasive infection caused by *Streptococcus dysgalactiae* ssp. *equisimilis*

emm type	No. of isolates (n = 231)	Poor outcome (n = 27)
stG6792[a]	55 (23.8)	11
stG485	37 (16.0)	1
stG6	21 (9.1)	4
stG10	21 (9.1)	2
stG652	17 (7.4)	1
stG2078	16 (6.9)	2
stC36	15 (6.5)	1
stG245	13 (5.6)	2
stG5420	6 (2.6)	1
stG480	5 (2.2)	1
stC6979	5 (2.2)	0
stG4974	4 (1.7)	1
stG653	3 (1.3)	0
Other	11 (4.8)	0
Non-typeable	2 (0.9)	0

[a]This *emm* type includes stG6792.3 (n = 54) and stG6792.0 (n = 1).

49 (n = 12), 89 (n = 5), 11 (n = 4), 12 (n = 4), and 75 (n = 4), whereas *emm* types 12 and 6 predominated in our study of non-invasive GAS infections (T. Wajima, S. Y. Murayama & K. Ubukata, unpublished data).

Capsular type Ib in GBS isolates were not associated with poor outcome of invasive diseases. This type (n = 39) was observed most frequently in adults. Other GBS capsular types, i.e. V (n = 23), II (n = 15), III (n = 14), and Ia (n = 11), were predominantly observed, showing distribution patterns different from those found in the study of non-invasive GBS infections.

Antibiotic susceptibility

Antibiotic susceptibility testing was performed for all isolates of SDSE, GAS and GBS. Of 231 SDSE isolates, four had the *mef*(A) gene, and 13 and six isolates had *erm*(A) and *erm*(B) genes, respectively; the presence of the latter suggested a high level of resistance to clarithromycin (MIC ≥64 mg/L). In addition, two SDSE isolates showed resistance to fluoroquinolones, such as levofloxacin (MIC ≥32 mg/L), based on substitutions in GyrA (Ser81 to Phe or Tyr) and ParC (Ser79 to Tyr). No penicillin or cephalosporin resistance was observed.

Discussion

To the best of our knowledge, this is the first nationwide surveillance regarding invasive infections caused by SDSE in Japan. The study demonstrates significant differences in clinical aspects, including prognosis, between disease entities caused by SDSE, GAS and GBS. The mortality rate of invasive SDSE diseases in our study (13%) was similar to those previously described in other countries (8–18%) [1,5,14,15].

Host-protective factors, including WBCs, platelets, and CRP, affect the severity and prognosis of infectious diseases, especially those involving invasive pathogens. Disturbed haemostasis is a central finding in severe *S. pyogenes* infection that is associated with M protein-induced platelet activation and thrombus formation [16]. In particular, microthrombi are found both at the local site of infection and at distant sites. Platelets are responsible for maintaining vascular function and haemostasis. With regard to WBCs and CRP, streptococcal erythrogenic toxin B induces apoptosis and proliferation in human leukocytes [17]. Moreover, serial CRP monitoring was found to alert physicians to complications and predict outcome earlier than clinical signs or roentgenograms in 63 children with acute haematogenous osteomyelitis [18]. In our investigations, a poor WBC response (<5000 cells/μL) and thrombocytopenia at admission each showed a significantly higher risk of poor outcome (death or invasive disease-associated sequelae) in patients with invasive SDSE or GAS infections. However, CRP could not predict poor outcomes. We need to continue to examine associations between prognosis and host defence factors.

The M protein of *S. pyogenes* is a major bacterial virulence factor that confers resistance to phagocytosis [19]. Recently, analysis of the *emm* gene, which codes for the amino acid sequence (variable region) at the N-terminal end of the M protein, has often been used for molecular epidemiological studies regarding outbreaks of invasive or non-invasive streptococcal infections [20,21]. Data suggesting horizontal gene transfer and recombination between the *emm* genes of SDSE and GAS strains have been observed in clinical isolates from seven subjects [21]. These genetic transfer and recombination events might have a role in pathogenesis. In our study, *emm* type stG6792 in SDSE isolates (n = 55), the type most frequently confirmed in SDSE infection, was significantly more often associated with poor outcome of invasive diseases than other SDSE *emm* types. In contrast to the high frequency in our study, only three strains of stG6792 were observed in a recent article from the USA [1]. Surveillance periods for strains differed between Japan (2006–2007) and the USA (2002–2004). On the basis of the CDC database concerning *emm* type sequences, the stG6792.3 reference strain appeared to be derived from a streptococcal isolate from India, suggesting that this strain might have spread from India to Japan. Similarly, the *emm1* type in GAS isolates (n = 27), the type that we found most frequently, was significantly related to poor outcome of invasive GAS infections. Relationships between *emm* types and prognosis of the SDSE and GAS infections in Japan should be investigated in an ongoing manner. In addition, we need to determine person-to-person transmission routes of SDSE, as the stG6792.3

strain (n = 54) was observed most frequently within the stG6792 type, and similar *Sma*I digestion patterns were found for the isolates using PFGE analysis.

Antibiotic susceptibility testing and resistance gene identification for SDSE isolates in our study revealed clarithromycin and levofloxacin resistance, as described by others [1,2,5]. On the other hand, susceptibility to β-lactam antibiotics, including penicillin and cephalosporins, was high, suggesting that they should be the usual drugs of choice.

Some limitations of our investigation should be noted. Broyles *et al.* [1] recently reported an annual incidence of invasive β-haemolytic streptococcal infections involving groups other than A and B in the USA of 3.17 cases per 100 000 persons. Our surveillance, on the other hand, was not a population-based study of the burden of infections caused by SDSE, GAS and GBS, as official, systemic surveillance has not yet been established in Japan. To our knowledge, however, our study is the largest record of cases of invasive illness caused by SDSE, GAS and GBS in Japan to date.

In our investigation, clinical aspects of invasive SDSE infections appear to differ from those caused by GBS, and to be somewhat more similar to those caused by GAS. These observations might be accounted for by our findings that genes encoding virulence factors (e.g. M protein) in SDSE could be partially shared with those in GAS, on the basis of results of whole genome analyses of original SDSE isolates. Moreover, clinical isolates of SDSE possessing group A antigen have been reported [7]. SDSE has been established as a possible component of the normal flora of the skin, oropharynx, and gastrointestinal and genitourinary tracts. We identified SDSE in respiratory tract specimens from patients with non-invasive SDSE diseases in another study (K. Sunaoshi, S. Y. Murayama & K. Ubukata, unpublished data). However, questions persist as to how SDSE invades deep tissues and vessels. Further investigations to clarify this issue are needed.

In conclusion, primary-care doctors, particularly those treating patients in emergency departments, should consider invasive diseases caused by SDSE, especially when treating elderly subjects with underlying medical conditions.

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Transparency Declaration

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The Effects of a PPARα Agonist on Myocardial Damage in Obese Diabetic Mice With Heart Failure

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SUMMARY

Recent studies have confirmed that PPARα agonists have not brought the anticipated benefits to patients with type 2 diabetes and potentially fatal heart disease. We hypothesized that such agonists may have a cardio-suppressive effect in treating such disorders, therefore, we inoculated diabetic KKAy mice with encephalomyocarditis virus (EMCv) to induce a diabetic model with severe myocardial damage. WY14643, a potent PPARα agonist, was administered intraperitoneally either simultaneously (WY14643-late group) or 3 days before viral inoculation (WY14643-early group). WY14643-treated mice, especially those in the WY14643-early group, had higher mortality than those in the vehicle-treated group (vehicle) in the first 5 days after EMCv inoculation. However, the survival rate in the vehicle group decreased rapidly after day 4 and was the lowest of all 3 groups by day 9. The WY14643-treated mice showed reduced body weight and blood glucose, improved myocardial pathological changes, lower cardiac TNF-α expression, and significantly higher adiponectin expression, whereas the LW/LC ratio was lower and cardiac UCP3 mRNA expression higher in the WY14643 treatment groups than in the vehicle group on day 4. WY14643 therefore has cardioprotective and cardio-suppressive effects when used to treat EMCv-induced myocarditis in diabetic mice. The cardioprotective effect may be due to its anti-inflammatory properties and its ability to increase cardiac adiponectin expression, whereas the reduced cardiac efficiency may be due to its enhancement of cardiac UCP3 mRNA expression. (Int Heart J 2010; 51: 199-206)

Key words: Tumor necrosis factor-α (TNF-α), Adiponectin, Uncoupling protein 3 (UCP3)

Peroxisome proliferator-activated receptorα (PPARα) belongs to the nuclear receptor superfamily and is known to regulate the expression of genes for the transport proteins and enzymes that participate in inflammation and metabolism.[1)] It has been shown that PPARα agonists are capable of decreasing the production of some inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) in cardiac myocytes.[2)] Moreover, the potent PPARα agonist WY14643 can directly increase the expression of adiponectin receptors in white adipose tissue, which bind with adiponectin and exert antidiabetic, antiatherosclerotic, and anti-inflammatory effects.[3)] Likewise, recent reports have indicated that PPARα agonists can improve the survival rate of experimental animals with heart failure.[4)]

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, however, found that PPARα agonists had not brought the anticipated benefits to heart failure in type 2 diabetic patients and had even increased the death rate due to fatal cardiac disease, although to a statistically insignificant degree,[5)] thus suggesting that PPARα agonists may have some adverse effects on myocardial damage or cardiac function despite their cardioprotective effects.

Uncoupling proteins (UCPs) are inner mitochondrial membrane proteins that play a role in dissipating the mitochondrial proton gradient by allowing protons to re-enter the mitochondrial matrix without the concomitant synthesis of ATP. Three such proteins—UCP1, UCP2, and UCP3—are known.[6)] We are interested in UCP3 because it is mainly expressed in heart and skeletal muscle and is involved in the regulation of biological processes associated with mitochondrial energy metabolism.[7)] Indeed, increased UCP3 levels have been reported to correlate with higher myocardial consumption and reduced cardiac efficiency.[8)] WY14643 can increase the level of UCP3 mRNA in liver and brown fat tissue of KKAy mice.[3)] Likewise, myocardial levels of UCP3 in BALB/c mice increased by 54% upon treatment with WY14643.[9)] WY14643 may therefore exacerbate heart failure by increasing UCP3 expression, although more evi-

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dence is needed in the event of diabetes complicated with fatal heart disease.

Some metabolic diseases, such as type 2 diabetes and obesity, predispose to heart failure, and inflammation plays an important role in the association between them.[10] Encephalomyocarditis virus (EMCv) can induce severe myocarditis and heart failure in experimental animals.[11] In previous studies, we inoculated EMCv into obese mice to set up a model of obese mice with severe heart failure, and found higher TNF-α and lower adiponectin expression levels in the injured myocardium.[12,13] Herein, obese diabetic KKAy mice inoculated with EMCv were used to induce a model of type 2 diabetes and obesity complicated with severe heart failure, and subsequently to evaluate the effect of WY14643. We determined the survival rate and examined the expression levels of cardiac TNF-α, adiponectin, and UCP3 at different stages of myocardial damage and found that WY14643 plays different roles in the injured heart.

METHODS

Animals and treatments: Eight-week-old female KKAy mice weighing 38-42 g were purchased from Clea Japan Inc. (Tokyo), and maintained with food and water *ad libitum*. These mice were randomly divided into 3 groups: a WY14643-early group, which received WY14643 (Sigma, USA) at a daily dose of 50 mg/kg starting 3 days before viral inoculation, and a WY14643-late group and vehicle treatment group (vehicle), which received WY14643 and vehicle (dimethyl sulfoxide), respectively, simultaneously with viral inoculation. WY14643 (0.1 mL) was administered intraperitoneally once daily. Experimental protocols were approved by the Animal Experimental Committee of Kanazawa Medical University.

Study design: The study consisted of two experiments. Experiment 1 was performed to determine the survival rate and any change in body weight (BW). In this experiment, 21, 28, and 19 mice were raised in the vehicle, WY14643-early, and WY14643-late groups, respectively. The survival rate and BW were recorded daily from 3 days before EMCv inoculation. The endpoint was a survival rate of less than 20% in any group. Experiment 2 was designed to obtain plasma and tissue samples on days 0, 4, and 9 after EMCv inoculation, with 45 mice in the vehicle group, 39 in the WY14643-early group, and 18 in the WY14643-late group. Eight KKAy mice without inoculation and treatment were used as normal control (control) and sacrificed on day 0.

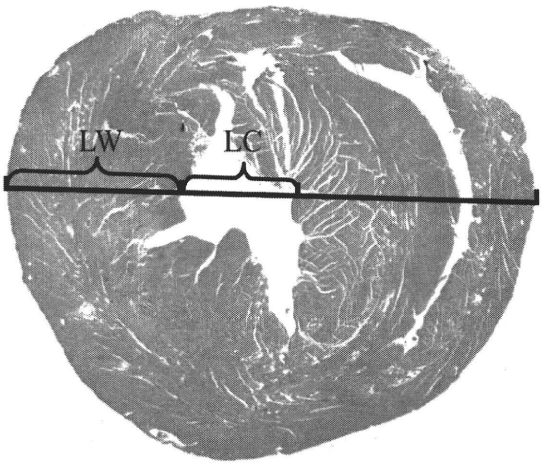

Figure 1. Measurement of left ventricular wall thickness and cavity dimension. LW indicates left ventricular wall and LC, left cavity.

EMCv and inoculation protocol: A myocarditic variant of EMCv was provided by Dr. Y. Seto, Institute for Advanced Medical Research of Keio University (Tokyo). Virus preparations and inoculation were performed as described previously.[13]

Histological heart examinations: The BW and heart weight (HW) of each mouse was recorded at sacrifice in experiment 2. The heart was transversely divided into two halves. One half, which included the cardiac apex, was stored at -80°C until use, while the other half was fixed in 10% buffered formalin and blocked with paraffin for histological examination and immunohistochemical study. After fixation, the heart was transversely sectioned at the maximal circumference of the ventricle. Some slices were stained with hematoxylin-eosin to assess the severity of myocardial necrosis and inflammatory cell infiltration according to previously described scales.[13,14]

The LV wall thickness (LW) and LV cavity (LC) dimensions were measured with the slice at the maximal circumference of the ventricle, as described by Matsumori, *et al*[15] (Figure 1). The diameter of the myocardial fiber in the lateral LV wall was determined as described previously.[13]

Blood glucose (BG) and plasma free fatty acid (FFA): BG and plasma FFA were determined with a Fuji Dry Chem System (Medical System Co., Tokyo) and nonesterified fatty acid-C test kit (Wako Pure Chemical Industries, Osaka, Japan), respectively.

Immunohistochemical examination: To identify the anatomic localization and expression levels of TNF-α, adiponectin, and UCP3 within the myocardium, immunohistochemistry was performed using the avidin biotin complex methods (Vectastain ABC kit, Vector Laboratories, Burlingame, CA), as described previously.[14] All sections were previously blocked with normal goat serum for 20 minutes at room temperature to minimize background staining. The slides were then incubated with anti-human TNF-α mouse monoclonal antibody clone 4H31 (#HM2010, HyCult Biotechnology b.v., Uden, the Netherlands), anti-human adiponectin mouse monoclonal antibody clone (#ab22554, Abcam, Tokyo), and UCP3 rabbit polyclonal antibody #ab10985, Abcam, Japan). Sections were counterstained with hematoxylin. The slides were reviewed blind by the same pathologist and graded semiquantitatively according to the degree of immunoreactivity: 0 for absence of staining, 1 for weak, 2 for moderate, and 3 for strong staining.[16] They were then compared with the respective control slides to exclude nonspecific staining.

Comparative expression of TNF-α, adiponectin, and UCP3 mRNA in heart tissue: RNA extraction from the frozen cardiac tissue was performed following the manufacturer's protocol (RNeasy Mini Kit, QIAGEN Inc., Tokyo). DNAase was applied during RNA extraction to avoid DNA

contamination. Total RNA concentration was determined by measuring the optical density at 260 and 280 nm. Aliquots of 20 μL RNA from each tissue sample were used to produce cDNA. Comparative expression levels of TNF-α, adiponectin, and UCP3 mRNA in cardiac tissue from each group were determined using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR). A TaqMan minor groove binding (MGB) Probe (Applied Biosystems Inc., CA, USA) was applied for real-time PCR, and a commercially available kit was used for TNFα, adiponectin, and UCP3 RT-PCR (Applied Biosystems Inc.). Primers and TaqMan probes for the target gene (TNFα, adiponectin, and UCP3) and internal reference gene (Rodent GAPDH) were purchased from Applied Biosystems (TaqMan® Gene Expression Assays). Each TaqMan probe was labeled with a reporter dye [6-carboxyfluorescein (FAM)] situated at the 5' end of the oligonucleotide and a quencher dye (MGB) located at the 3' end. TaqMan® Gene Expression Assay numbers for TNF, adiponectin, and UCP3 were Mm00443258mL, Mm00456425mL, and Mm00494074mL, respectively (Applied Biosystems Inc.). Quantification of target cDNA (TNFα, adiponectin, and UCP3) and GAPDH was performed in 96-well plates using an ABI PRISM7500 Sequence Detection System (ABI); data collection and analysis was performed using the machine's software. PCR was carried out on a final volume of 25 μL containing cDNA equivalent to 10-100 ng of total RNA, 10 μL of 2 × TaqMan Fast PCR Master Mix, and 1 μL of 20 × TaqMan Expression Assay reagent. Each sample was analyzed in triplicate. The thermal cycler conditions were 95°C for 20 seconds, followed by 40 cycles at 95°C for 3 seconds, and 60°C for 30 seconds. The comparative C_T method of data analysis was used to analyze the data. C_T is the PCR cycle at which an increase in reporter fluorescence above the baseline level was first detected. C_T values for the target and internal reference gene were calculated for each sample along with the difference between these values (ΔC_T). $\Delta\Delta C_T$ was calculated as the difference in ΔC_T between sample and calibrator sample. The amount of target gene expression, normalized to an internal reference and relative to calibrator, was given by: $2^{-\Delta\Delta CT}$.

Statistical analysis: The Kaplan-Meier analysis and a log rank test were used to assess the survival rate of mice in each group. Other data are expressed as the mean ± SEM and were analyzed by ANOVA. When results were found to be significant, comparisons were performed using the Bonferroni test. Statistical significance was defined as $P < 0.05$.

Results

Survival rate and BW in experiment 1: According to the Kaplan-Meier analysis, WY14643-treated mice, especially those in the WY14643-early group, had higher mortality than those in the vehicle group in the first 5 days after EMCv inoculation, although the survival rate in the vehicle group decreased rapidly after day 4 and was the first to drop

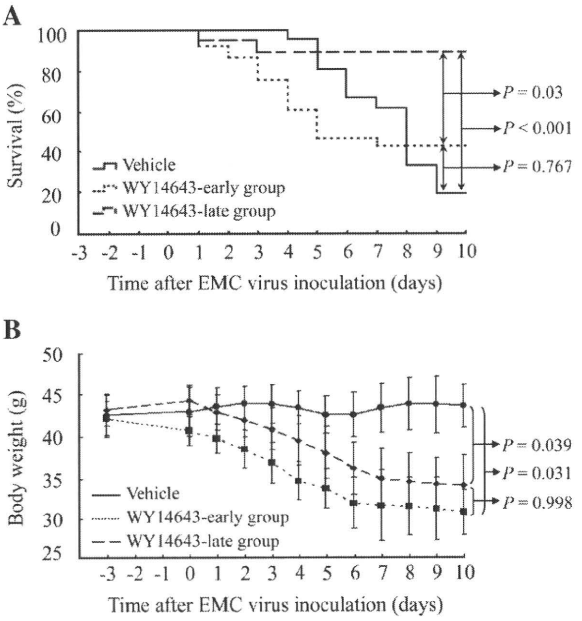

Figure 2. A: Results of the Kaplan-Meier survival analysis showing the lower mortality during the later stages, and the higher mortality during the earlier stages, of myocarditis in the WY14643 treatment groups than in the vehicle group. **B:** Treatment with WY14643 decreased the body weight of KKAy mice significantly.

Table I. Effects of WY14643 on Body Weight (BW), Heart Weight (HW), Blood Glucose (BG), and Plasma Free Fatty Acid (FFA) in KKAy Mice With Acute Viral Myocarditis

	n	BW (g)	HW (mg)	HW/BW (%)	BG (mmol/L)	FFA (mEq/L)
Day 0						
Control	8	38.4 ± 2.0	152.7 ± 16.8	0.40 ± 0.02	7.07 ± 0.18	1.05 ± 0.12
WY14643-early	8	35.4 ± 2.1$^{\#}$	132.0 ± 7.5$^{\#}$	0.37 ± 0.03	3.63 ± 1.05$^{\#\#}$	1.19 ± 0.03$^{\#}$
Day 4						
Vehicle	8	38.0 ± 4.4	174.3 ± 7.1	0.46 ± 0.05	14.13 ± 4.74	1.43 ± 0.05
WY14643-early	8	33.1 ± 2.0*	119.5 ± 10.1**	0.36 ± 0.03*	1.81 ± 0.49**	1.21 ± 0.09*
WY14643-late	8	34.8 ± 0.6*	143.3 ± 27.5*	0.41 ± 0.08	2.28 ± 1.04**	1.80 ± 0.06*†
Day 9						
Vehicle	7	43.6 ± 2.2	153.4 ± 13.0	0.35 ± 0.02	10.66 ± 2.09	1.84 ± 0.10
WY14643-early	9	33.0 ± 3.3**	114.3 ± 9.1**	0.35 ± 0.01	6.09 ± 1.17**	1.47 ± 0.13**
WY14643-late	8	36.0 ± 3.6*	141.5 ± 11.1*	0.40 ± 0.05	7.32 ± 2.79*	1.48 ± 0.16**

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ with respect to control; $^{*}P < 0.05$, $^{**}P < 0.01$ with respect to vehicle on the corresponding day; $^{\dagger}P < 0.01$ with respect to WY14643-early group.