

the early stages of the disease process, while albumin excretion was still in the normal range [25]. Thus, it may serve as early marker for tubular damages in diabetic nephropathy and may precede albumin excretion to the urine [25–27]. Low molecular weight markers of tubular dysfunction such as α 1-microglobulin, therefore, appear as markers of renal dysfunction that may complement markers of glomerular dysfunction such as albumin [7]. Orosomucoid, α -1 acid glycoprotein with 41 kDa, is an acute-phase protein and the serum concentrations correlated with low-grade inflammation in the patients with diabetes [28,29]. Urine excretion of orosomucoid was increased in the patients with diabetes and normoalbuminuria and it correlated with markers of inflammation such as CRP [29–31] and markers for endothelial dysfunction [32]. Type 2 diabetic patients with elevated urinary orosomucoid excretion exhibited normal glomerular and tubular function, suggesting the possibility of local renal production of orosomucoid due to chronic low-grade inflammation rather than hyperfiltration [33].

Fetuin-A has been principally studied as an inhibitor for ectopic calcium deposition in the renal field and it is also an important promoter for insulin resistance. Fetuin-A, a liver secretory glycoprotein with 64 kDa, has been shown that it acts as carrier of free fatty acids (FFAs) and they are the intrinsic ligands for Toll-like receptor 4 (TLR4), which induces adipose tissue inflammation and insulin resistance [34]. Fetuin-A binds to the residues of Leu100-Gly123 and Thr493-Thr516 of TLR4 through the terminal galactoside moiety [34]. Thus, FFA-Fetuin-A induced TLR4 activation is very important in the lipid-induced inflammation and insulin resistance and type 2 diabetes. In addition, fetuin-A and adiponectin mediate the crosstalk between adipose tissues, liver and kidney. Fetuin-A suppresses mRNA expression of adiponectin in cultured human adipocytes and treatment of wild-type mice with fetuin-A lowered serum adiponectin levels [35]. Higher fetuin-A and lower adiponectin levels may contribute the development of insulin resistance, diabetes and subsequent obesity-related CKD and diabetic nephropathy [36]. Serum concentration of fetuin-A in type 2 diabetes patients has been reported and they positively correlated with macrovascular late complications in high-risk type 2 diabetes patients, while no association with metabolic status or microvascular complications [37]. Recent study indicated that serum fetuin-A is lower in microalbuminuria patients compared with normo- and macroalbuminuric patients [38]. In other studies, lower serum levels of fetuin-A are associated with peripheral arterial disease in patients with type 2 diabetes [39] and serum fetuin-A levels are negatively associated with atherosclerotic calcified plaques [40]. Thus, the significance of serum fetuin-A levels is controversial whether it is a good marker for diabetic micro- and macrovascular complications. Unfortunately, we failed to detect changes in binding activities to various lectins in the sera from the patients with various stages of diabetic nephropathy (data not shown), we did not get a chance to measure the serum levels of fetuin-A. However, we demonstrated

that the urinary excretion of fetuin-A is a candidate for the biomarker to predict the progression of diabetic nephropathy. Although two previous published studies identified fetuin-A in urine samples of the patients with diabetic nephropathy, the quantifications were limited to inaccurate estimations by fluorescence 2-D differential in-gel electrophoresis [41] and capillary electrophoresis coupled to mass spectrometry [42]. In contrast to previous studies, we firstly used stable ELISA kit to quantify the urinary excretion of fetuin-A. Urinary concentration of fetuin-A may be depending on the production from the liver, alterations in permeability through glomerular basement membrane by capillary damages and changes in tubular reabsorption. Higher excretion of fetuin-A into urine has been reported to reflect the insulin resistance and inflammatory responses in obesity and type 2 diabetes [43] and it may reflect the increase in the serum levels of fetuin-A and alterations in the changes in the permeability of glomerular capillaries. Fetuin-A is reported to pass through the slit diaphragm and re-introduced to proximal tubular cells by megalin-mediated endocytosis [44]. Zhou et al. also reported that urinary exosomal fetuin-A was increased in the rats treated with cisplatin injection and in the ICU patients with acute kidney injury [45]. Thus, an alternative explanation for increased urinary fetuin-A excretion in diabetic nephropathy could be due to the tubular injury. In our study, multivariate regression analysis indicated that higher urinary fetuin-A excretion demonstrated a higher risk for the development of microalbuminuria and reduction of renal function and future cohort study is required to further confirm this notion.

Conclusions

In summary, the glycan profiling studies using urine samples from the patients with diabetic nephropathy is useful to identify the new biomarkers to predict the progression of diabetic nephropathy. We demonstrated that global reduction of the bindings to lectins, such as fucose, Lac/LacNA, α - or β -Gal, chitobiose, and α - or β -GalNAc binders in urine samples of diabetic nephropathy at macroalbuminuria stage, and in contrast increased binding activity to Sia α 2-6-Gal/GalNAc binders. Further, we identified three sialylated glycoproteins such as α 1-microglobulin (Protein AMBP), α 1-acid glycoprotein (orosomucoid) and fetuin-A (α 2-HS-glycoprotein) by SSA-Agarose column chromatography and LC/MS-MS analysis. Finally, we have newly shown that higher urinary excretion of fetuin-A is a risk factor for both microalbuminuria and reduction of GFR in diabetic nephropathy.

Author Contributions

Wrote the paper: JW KI HM. Designed and performed most of the experiments: KI JW. Recruited the patients: JE AN ST KM DO TT AK AT II KH. Performed lectin microarray analysis: MY TO. Conceived the study: JW KI HM.

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Combination therapy with an angiotensin-converting-enzyme inhibitor and an angiotensin II receptor antagonist ameliorates microinflammation and oxidative stress in patients with diabetic nephropathy

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ABSTRACT

Aims/Introduction: Recent studies have pointed to the effectiveness of combination therapy with an angiotensin-converting-enzyme inhibitor (ACEI) and an angiotensin receptor blocker (ARB) for diabetic nephropathy. However, some controversy over this combination treatment remains and the mechanisms underlying its renoprotective effects have not been fully clarified. Therefore, we compared the renoprotective effects of imidapril (ACEI) and losartan (ARB) combination therapy with losartan monotherapy in patients with diabetic nephropathy. We also compared the anti-inflammatory and anti-oxidative stress effects of these two treatments.

Materials and Methods: A total of 32 Japanese patients with type 2 diabetes and nephropathy were enrolled. Patients were randomized to either 100 mg/day losartan ($n = 16$) or 50 mg/day losartan plus 5 mg/day imidapril ($n = 16$). We evaluated clinical parameters, serum concentrations of high-sensitivity C-reactive protein (hs-CRP), soluble intercellular adhesion molecule-1 (sICAM-1), interleukin-18 (IL-18) and monocyte chemoattractant protein-1 (MCP-1), and the urinary concentrations of IL-18, MCP-1 and 8-hydroxy-2'-deoxyguanosine (8-OHdG) at 24 and 48 weeks after starting treatment.

Results: Blood pressure was not significantly different between the two groups. The serum levels of hs-CRP, sICAM-1 and IL-18, as well as urinary excretion of albumin, IL-18 and 8-OHdG decreased significantly in the combination therapy group at 48 weeks. The percent decreases in serum IL-18 concentrations and urinary IL-18 and 8-OHdG were significantly greater in the combination therapy group than in the monotherapy group.

Conclusions: Combination therapy with an ACEI and an ARB could be beneficial for treating diabetic nephropathy through its anti-inflammatory and anti-oxidative stress effects. (*J Diabetes Invest* doi: 10.1111/jdi.12004, 2013)

KEY WORDS: Combination, Diabetic nephropathy, Renin–angiotensin system

INTRODUCTION

It is widely accepted that chronic inflammation is profoundly involved in the development of atherosclerosis¹. Adhesion molecules, pro-inflammatory cytokines and chemokines, including soluble intercellular adhesion molecule-1 (sICAM-1), inter-

leukin-18 (IL-18) and monocyte chemoattractant protein-1 (MCP-1), are involved in the pathogenesis of diabetic nephropathy as well as atherosclerosis^{2,3}. C-reactive protein (CRP) is a good marker for cardiovascular risk⁴, and is a precipitating factor for diabetic nephropathy⁵. Angiotensin II, which is produced by the renin–angiotensin system (RAS), is known to promote inflammation⁶. Inhibition of the RAS and associated inflammation might be renoprotective in chronic renal diseases, including diabetic nephropathy^{6,7}.

Oxidative stress is a critical pathogenic component of atherosclerosis and diabetic nephropathy⁸. After the onset of renal disorders, the levels of pro-inflammatory cytokines and oxidative stress begin to increase, inducing cardiovascular diseases through vascular endothelial dysfunction⁹. Furthermore, an increase in

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oxidative stress has been reported in hyperglycemic rats¹⁰. While activation of the RAS increases oxidative stress, angiotensin-converting enzyme inhibitors (ACEI)¹¹ and angiotensin II type 1 receptor blockers (ARB)¹² inhibit oxidative stress.

Combination therapy with an ACEI and an ARB has been considered in several renal diseases to protect the kidney by potently inhibiting RAS activity¹³. In the context of diabetic nephropathy^{6,13,14}, combination therapy was found to reduce albuminuria. However, most of the studies testing ACEI/ARB combination therapy in diabetic nephropathy were short-term observational studies, and clinical studies attempting to elucidate the mechanisms underlying these renoprotective effects are not sufficient¹⁵.

In patients with diabetic nephropathy, ACEI/ARB combination therapy is expected to have more potent anti-inflammatory and anti-oxidative stress effects than monotherapy at the systemic and local levels in the kidney. Combination therapy might also inhibit the development or progression of atherosclerosis and diabetic nephropathy more potently than monotherapy. However, to our knowledge, no clinical studies have evaluated the anti-inflammatory and anti-oxidative stress effects of combination therapy. Therefore, we carried out a randomized controlled study of ACEI/ARB combination therapy vs ARB monotherapy in patients with type 2 diabetes and early nephropathy to compare the anti-inflammatory and anti-oxidative stress effects of these therapies.

MATERIALS AND METHODS

Study Design

Patients meeting the following inclusion criteria were eligible for the present study: age 30–74 years, diagnosed with type 2 diabetes, disease duration ≥ 7 years, diagnosis of diabetic neuropathy and retinopathy, creatinine clearance (C_{Cr} ; determined using the Cockcroft–Gault formula) >60 mL/min and urinary albumin/creatinine ratio (ACR) >30 mg/gCr. The diagnosis of type 2 diabetes was made according to the World Health Organization criteria.

Patients meeting any of the following criteria were excluded from the present study: patients with chronic inflammatory disease or malignancies, pregnant women or women who wished to become pregnant, patients with renovascular hypertension, patients with hemoglobin A1C (A1C; National Glycohemoglobin Standardization Program) $>9.4\%$; patients with blood pressure (BP) $>180/110$ mmHg and patients who had participated in another clinical trial within 3 months before enrolling in the present study. At the discretion of the investigator, patients who had previously used an ACEI or ARB that could not be washed out were also excluded. Concomitant use of steroids, potassium-sparing diuretics, digoxin, or antiarrhythmic drugs except for β -blockers and calcium channel blockers was not allowed. Concomitant use of new statins or thiazolidinedione derivatives was not allowed during the washout or observation periods. Blood glucose levels were to be controlled by adjusting the dose of medications already in use.

Written informed consent was obtained from all participants. The present study was approved by the Ethics Committee of Okayama Saiseikai General Hospital.

The design of the present study is summarized in Figure 1a. During the washout period, previous ACEIs or ARBs were discontinued and switched to amlodipine (5 mg/day). The observation period was started 8 weeks after starting treatment with amlodipine. In the present study, we treated patients with losartan (ARB) and imidapril (ACEI) or losartan alone during the observation period. Amlodipine was stopped when starting combination or monotherapy.

For losartan monotherapy, the losartan dose was 100 mg/day. In combination therapy, the doses of losartan and imidapril were 50 and 5 mg/day, respectively. The first patient enrolled in the study was allocated to monotherapy, with subsequent patients allocated to one of the two therapies.

During the observation period, patients were to be discontinued or withdrawn from the study if BP could not be maintained at $<180/110$ mmHg, C_{Cr} decreased to 60 mL/min, serum creatinine increased from baseline by $\geq 30\%$, or if an adverse drug reaction possibly related to therapy occurred.

Data Collection

After the baseline visit, patients were instructed to visit the hospital at 8, 16, 24 and 48 weeks. At each visit, physical findings, BP, hematological parameters, urinalysis parameters and complications were assessed (Figure 1b). Venous blood and urine samples were obtained in the early morning after an overnight fast. BP was measured by the same person in the morning. After a 5-min rest in a sitting position, BP was measured in triplicate to calculate the mean for assessment.

Analysis of Biomarkers

The primary objective of the present study was to evaluate the anti-inflammatory and anti-oxidative stress effects of imidapril/losartan combination therapy by measuring the serum concentrations of high-sensitivity CRP (hs-CRP), sICAM-1, IL-18, MCP-1 and aldosterone, as well as the urinary concentrations of IL-18, MCP-1, aldosterone and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Blood and urine samples were centrifuged immediately after collection, and the supernatants were stored at -80 and -30°C , respectively, until analysis. The concentrations of the pro-inflammatory biomarkers were analyzed using samples obtained at baseline and at weeks 24 and 48. All samples were measured together once all specimens were collected.

An immunoturbidimetric assay was used for urinary albumin concentrations (Nitto Boseki Co. Ltd, Tokyo, Japan) and an immunonephelometric assay kit was used for hs-CRP concentrations (Dade Behring, Marburg, Germany). Enzyme-linked immunosorbent assay kits were used to measure IL-18 (MBL, Nagoya, Japan), sICAM-1 concentration and MCP-1 concentrations (R&D Systems, Inc, Minneapolis, MN, USA), and 8-OHdG concentration (Nikken SEIL Co. Ltd, Tokyo, Japan). A radioimmunoassay kit was used to measure aldosterone

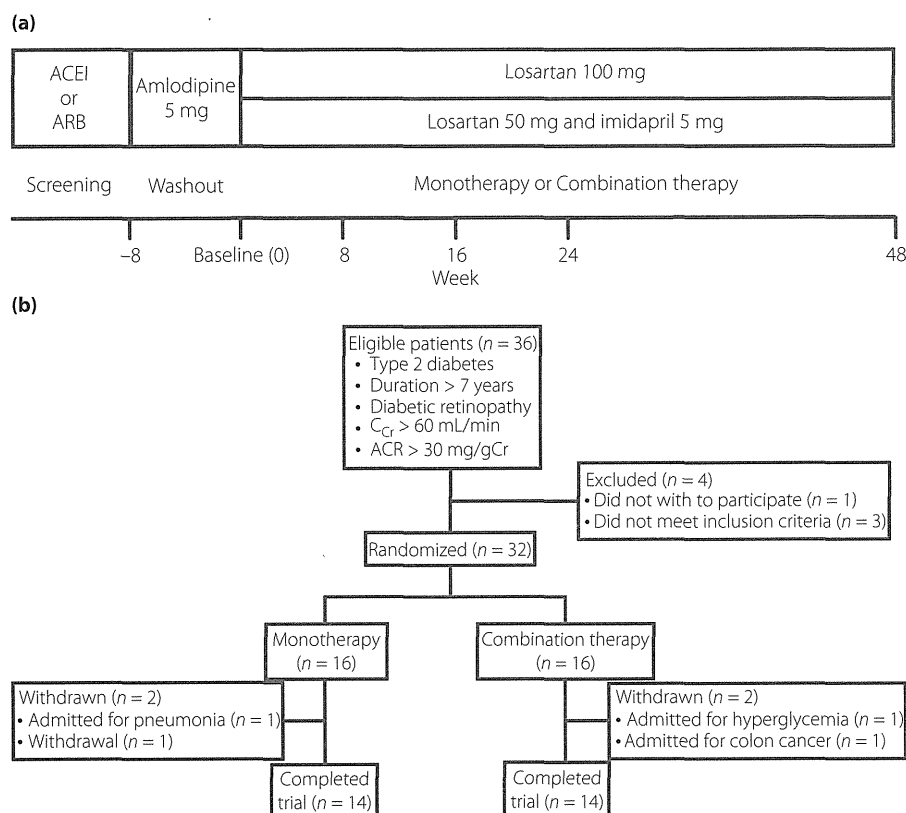


Figure 1 | (a) Study design. During the washout period, angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) were discontinued and switched to amlodipine (5 mg/day). At the end of the washout period, patients were allocated to either monotherapy (100 mg/day losartan) or combination therapy (50 mg/day losartan plus 5 mg/day imidapril) and followed for 48 weeks. (b) Patient disposition. Four patients were excluded from this study during the washout period, including one patient who refused to participate in the study, two patients with an ACR < 30 mg/gCr and one patient with C_{Cr} < 60 mL/min. Of the 32 patients who started the observation period, 28 were treated for 48 weeks and were included in the analysis.

concentrations (TFB Co. Ltd, Tokyo, Japan). A1C was measured using a high-pressure liquid chromatography method and is expressed in National Glycohemoglobin Standardization Program values. The urinary markers were divided by the urinary creatinine concentration to calculate the creatinine ratio for assessment.

Statistical Analysis

Comparisons of baseline factors between the two therapeutic groups were analyzed by Student's unpaired *t*-test for continuous parameters or chi-squared-test for categorical parameters (sex and previous therapy). Baseline concentrations of pro-inflammatory markers, serum and urinary aldosterone concentrations, and urinary 8-OHdG concentrations were compared using Student's unpaired *t*-test. The percent change (% change) from baseline for pro-inflammatory biomarkers, serum and urinary aldosterone concentrations, urinary 8-OHdG concentration, A1C, BP, and C_{Cr} at 24 and 48 weeks was calculated and analyzed by analysis of variance (ANOVA). The differences in the percent change of these parameters at each time between the

two therapeutic groups were analyzed by ANOVA. All analyses were two-sided with a significance level of 5%. Data are expressed as the number for categorical variables, mean change from baseline for percent changes and mean \pm standard deviation for continuous variables.

RESULTS

A total of 36 patients who met the inclusion criteria were screened (Figure 1b). Before screening, all of the patients had received an ACEI and/or ARB. Four patients were excluded from the study during the washout period, including one patient who refused to participate in the study, two patients with an ACR < 30 mg/gCr and one patient with C_{Cr} < 60 mL/min. Therefore, 32 patients started the study. In two patients allocated to combination therapy, the doses of losartan and imidapril were 25 and 2.5 mg/day, respectively, for the first 4 weeks because their systolic BP (SBP) was <120 mmHg. The doses in these patients were subsequently increased to 50 and 5 mg/day, respectively. During the observation period, two patients allocated to combination therapy were

excluded from the study; one patient was hospitalized for hyperglycemia and one patient underwent surgery for colon carcinoma. In the monotherapy group, two patients were excluded during the observation period; one patient was hospitalized for pneumonia and one patient stopped visiting the hospital. Consequently, 14 patients in each therapeutic group, a total of 28 patients, completed 48 weeks of observation and were included in the analysis (Table 1). There were no significant differences in baseline parameters between the two therapeutic groups, including age, sex, duration of diabetes, body mass index, A1C, Ccr, SBP, diastolic BP (DBP), ACR and previous therapy.

None of the patients developed new cerebrovascular disease, cardiovascular disease, arteriosclerosis obliterans, progression of nephropathy, excessive BP decrease or hyperkalemia during the study. Urinalysis showed no evidence of ketonuria, hematuria or urinary tract infection.

Time-Course of A1C, BP and C_{Cr}

There were no significant differences in percent change in A1C at 8, 16, 24 or 48 weeks between the monotherapy (7.8 ± 1.6, 8.0 ± 1.2, 8.0 ± 1.3 and 7.9 ± 1.2%, respectively) and combination therapy groups (7.8 ± 0.6, 8.1 ± 1.0, 8.1 ± 1.0 and 7.8 ± 0.8%, respectively). Similarly, there were no significant differences in SBP (monotherapy 137 ± 7, 136 ± 8, 135 ± 7 and 134 ± 8 mmHg; combination therapy 134 ± 12, 133 ± 10, 137 ± 10 and 134 ± 9 mmHg), DBP (monotherapy 80 ± 6, 79 ± 7, 81 ± 7 and 79 ± 5 mmHg; combination therapy 79 ± 8, 80 ± 8, 80 ± 8 and 78 ± 8 mmHg) or C_{Cr} (monotherapy 84.6 ± 18.6, 85.2 ± 20.4, 82.8 ± 18.6 and 84.6 ± 21.6 mL/min; combination therapy 85.2 ± 31.8, 87.6 ± 33.6, 86.4 ± 34.2 and 86.4 ± 30.6 mL/min) at 8, 16, 24 and 48 weeks of treatment.

Table 1 | Baseline background of patients in each group

	Mono	Combination
No. patients	14	14
Age (years)	61.4 ± 8.8	61.7 ± 5.3
Sex (male/female)	9/5	10/4
Duration of diabetes (years)	11.7 ± 4.7	15.6 ± 6.7
Body mass index (kg/m ²)	25.4 ± 2.9	24.5 ± 5.1
A1C (%)	7.8 ± 1.5	7.8 ± 0.8
Creatinine clearance (mL/min)	85.4 ± 16.1	90.1 ± 36.1
Systolic blood pressure (mmHg)	136 ± 6	134 ± 12
Diastolic blood pressure (mmHg)	79 ± 6	79 ± 7
Albumin creatinine ratio (mg/gCr)	224 ± 197	270 ± 202
Medication (n)		
Insulin	5	4
Oral hyperglycemic agents	5	9
Statins	5	4

A1C, hemoglobin A1C; Mono, monotherapy.

Data are n or mean ± standard deviation.

Time-Course of Serum Concentrations of Pro-Inflammatory Biomarkers and Aldosterone

At baseline, we found no significant differences in the serum concentrations of hs-CRP, sICAM-1, IL-18, MCP-1 or aldosterone between the two groups (Table 2).

In all patients, the serum hs-CRP concentration decreased significantly from baseline by 23.5% at 48 weeks. In the combination therapy group, the serum hs-CRP concentration decreased significantly from baseline by 28.0 and 31.0% at 24 and 48 weeks, respectively. The serum hs-CRP concentration decreased by 16.6% at 48 weeks in the monotherapy group, although not significantly (Table 2). The serum sICAM-1 concentration decreased significantly by 10.6% at 48 weeks in

Table 2 | Change of the serum levels of inflammatory molecules and aldosterone

Serum molecules	Week 0	Week 24	Week 48
hsCRP (mg/L)			
All patients	1.33 ± 0.77	1.04 ± 0.79	0.71 ± 0.60
% change		-13.7	-23.5*
Mono	1.39 ± 0.93	1.24 ± 1.01	1.04 ± 0.75
% change		-0.4	-16.6
Combination	1.26 ± 0.56	0.83 ± 0.39	0.78 ± 0.36
% change		-28.0*	-31.0‡
sICAM-1 (pg/mL)			
All patients	275 ± 76	256 ± 74	250 ± 54
% change		-5.9*	-6.4
Mono	282 ± 96	265 ± 100	262 ± 65
% change		-5.4	-2.1
Combination	269 ± 52	247 ± 35	238 ± 38
% change		-6.4	-10.6‡
IL-18 (pg/mL)			
All patients	195 ± 62	209 ± 89	194 ± 75
% change		+7.2	0.0
Mono	180 ± 49	214 ± 107	207 ± 73
% change		+16.4	+11.6
Combination	211 ± 72	204 ± 69	182 ± 53
% change		-2.0	-11.4*§
MCP-1 (pg/mL)			
All patients	308 ± 95	283 ± 78	282 ± 63
% change		-6.3*	-5.6*
Mono	331 ± 114	295 ± 84	291 ± 71
% change		-7.8	-8.0
Combination	285 ± 68	270 ± 74	273 ± 56
% change		-4.8	-3.2
Aldosterone (pmol/L)			
All patients	2470 ± 1180	2230 ± 930	1760 ± 950
% change		-5.4	-27.0‡
Mono	2680 ± 1220	2510 ± 840	1860 ± 1210
% change		+1.4	-31.2‡
Combination	2260 ± 1150	1960 ± 960	1650 ± 610
% change		-12.1*	-22.7‡

Data are means ± SD or frequencies (%). **P* < 0.05, †*P* < 0.01, ‡*P* < 0.005 versus baseline. §*P* < 0.05 for combination versus monotherapy (Mono).

the combination therapy group, but did not change significantly in the monotherapy group (Table 2). The serum IL-18 concentration decreased significantly by 11.4% at 48 weeks in the combination therapy group, but tended to increase in the monotherapy group, resulting in a significant difference in the percent change between the two groups at 48 weeks (Table 2). In all patients, the serum MCP-1 concentration decreased significantly from baseline by 6.3 and 5.6% at 24 and 48 weeks, respectively. The serum MCP-1 concentration decreased in both therapeutic groups, although the percent change was not significant. The serum aldosterone concentration decreased significantly from baseline at 48 weeks in all patients and in both groups (Table 2).

Time-Course of ACR and Urinary Concentrations of IL-18, MCP-1, Aldosterone and 8-OHdG

At baseline, there were no significant differences between the two groups in ACR or urinary concentrations of IL-18, MCP-1, aldosterone or 8-OHdG (Table 3).

In all patients, ACR decreased significantly by 8.3 and 11.0% at 24 and 48 weeks, respectively. ACR decreased significantly by 6.2% at 48 weeks in the monotherapy group and by 12.8 and 15.4% at 24 and 48 weeks, respectively, in the combination therapy group. The percent change (decrease) in ACR was significantly greater in the combination therapy group than in the monotherapy group at both times (Table 3). In all patients, the urinary IL-18 concentration tended to decrease over time, although not significantly. In the combination therapy group, the urinary IL-18 concentration decreased significantly by 55.3% at 48 weeks, and the percent change was significantly different from that in the monotherapy group (Table 3). In all patients, the urinary MCP-1 concentration decreased by 15.4% at 24 weeks, but increased significantly by 22.4% at 48 weeks. In both groups, the urinary MCP-1 concentration was increased at 48 weeks. The urinary aldosterone concentration was significantly decreased at all times in all patients and in both treatment groups; however, there were no significant differences in the percent changes between the two groups (Table 3). In all patients, the urinary 8-OHdG concentration decreased significantly from baseline by 28.6 and 26.6% at 24 and 48 weeks, respectively. The urinary 8-OHdG concentration decreased significantly by 23.8% at 24 weeks in the monotherapy group and by 33.5 and 37.8% at 24 and 48 weeks, respectively, in the combination therapy group. The percent change in 8-OHdG concentration at 48 weeks was significantly greater in the combination therapy group than in the monotherapy group (Table 3).

DISCUSSION

The present study showed that treatment with losartan and imidapril was more effective than treatment with amlodipine in decreasing ACR as well as inflammatory and oxidative stress markers in serum and urine in Japanese patients with type 2 diabetes and nephropathy. These effects were maintained for

Table 3 | Change of the urinary levels of albumin/creatinine ratio, inflammatory molecules, aldosterone and 8-hydroxy-2'-deoxyguanosine

Urinary molecules	Week 0	Week 24	Week 48
ACR (mg/gCr)			
All patients	252 ± 197	200 ± 202	175 ± 159
% change		−8.3‡	−11.0‡
Mono	234 ± 197	209 ± 221	182 ± 128
% change		−3.5	−6.2*
Combination	270 ± 202	192 ± 189	168 ± 191
% change		−12.8‡§	−15.4‡§
IL-18 (pg/mL/Cr)			
All patients	57 ± 62	33 ± 34	13 ± 14
% change		−15.7	−24.3
Mono	39 ± 35	25 ± 17	19 ± 17
% change		−7.3	+6.8
Combination	75 ± 77	40 ± 45	8 ± 8
% change		−24.1	−55.3‡§
MCP-1 (pg/mL/Cr)			
All patients	318 ± 192	205 ± 101	340 ± 244
% change		−15.4	+22.4*
Mono	341 ± 220	216 ± 109	351 ± 216
% change		−15.4	+32.8
Combination	294 ± 164	195 ± 95	329 ± 277
% change		−15.4	+12.0
Aldosterone (pmol/L/Cr)			
All patients	100 ± 70	48 ± 45	68 ± 54
% change		−50.9‡	−29.1‡
Mono	125 ± 79	68 ± 52	89 ± 59
% change		−35.7*	−22.6*
Combination	77 ± 68	29 ± 28	47 ± 41
% change		−65.1‡	−35.2
8-OHdG (pg/mL/Cr)			
All patients	12.8 ± 5.0	8.2 ± 4.2	8.6 ± 3.4
% change		−28.6‡	−26.6‡
Mono	13.7 ± 3.8	9.7 ± 4.3	10.7 ± 3.1
% change		−23.8*	−15.4‡
Combination	12.0 ± 5.9	6.8 ± 3.6	6.5 ± 2.3
% change		−33.5‡	−37.8‡§

Data are means ± SD or frequencies (%). * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.005$ versus baseline. § $P < 0.05$ for combination versus monotherapy (Mono).

48 week. Combination therapy with losartan and imidapril was more useful in decreasing ACR, and pro-inflammatory and oxidative stress markers than losartan administered at a double dose, without differences in BP or glycemic control. These results suggest that the losartan/imidapril combination might be useful to prevent the progression of atherosclerosis and nephropathy in patients with type 2 diabetes by exerting anti-inflammatory and anti-oxidative effects through inhibition of the RAS.

The RAS and downstream inflammatory activities are involved in the progression of atherosclerosis¹⁶. In the present study, treatment with losartan and imidapril did not significantly change A1C or BP, but hs-CRP levels at 24 and

48 weeks, and sICAM-1 levels at 48 weeks were significantly lower than those at baseline; these changes were not observed in the monotherapy group. Amlodipine was reported to have anti-inflammatory and anti-oxidative stress effects, and to inhibit the production of hs-CRP and sICAM-1^{17,18}. RAS inhibitors also have anti-inflammatory effects, which are favorable cardiovascular effects, independent of their antihypertensive effects^{19,20}. The present study showed that losartan and imidapril could inhibit the progression of atherosclerosis by their anti-inflammatory effects through inhibition of the RAS more potently than amlodipine.

The serum IL-18 concentration tended to be increased in the monotherapy group, but decreased significantly in the combination therapy group, resulting in a significant difference between the two groups at 48 weeks. Serum IL-18 is an important prognostic predictor of diabetic nephropathy and atherosclerosis²¹. It has been reported that ARBs and ACEIs decrease IL-18 by inhibiting the RAS²². In the present study, amlodipine was only administered during the washout period. Therefore, the efficacy of losartan and imidapril represented the change from treatment with amlodipine. Based on our searches of the literature, amlodipine has no known effect on serum IL-18 concentrations. These results indicate that imidapril/losartan combination therapy could have better anti-inflammatory efficacy in patients with type 2 diabetes with nephropathy, as compared with amlodipine or losartan monotherapy.

Similar to microinflammation, oxidative stress is profoundly involved in the development of atherosclerosis and diabetic complications²³. 8-OHdG is a biomarker of systemic DNA damage in diabetic nephropathy²⁴. In the present study, the urinary 8-OHdG concentration decreased significantly after treatment with losartan and imidapril, as compared with baseline levels. The decrease in urinary 8-OHdG concentrations was greater in the combination therapy group than in the monotherapy group. It has been reported that amlodipine^{17,18}, ACEI¹¹ and ARB^{12,19} suppress oxidative stress. However, it is unclear which of these agents or which combination of these drugs elicits the greatest reduction in oxidative stress in patients with diabetic nephropathy. The present results suggest that imidapril/losartan combination therapy might be more effective in preventing the development or progression of diabetic nephropathy because of its more potent inhibitory effects on oxidative stress than losartan monotherapy. These effects were maintained for up to 48 weeks.

The urinary aldosterone concentration was also decreased more markedly by combination therapy than monotherapy in the present study, which suggests that combination therapy more potently inhibits the RAS in the kidney. The urinary IL-18 concentration was also significantly decreased by combination therapy as compared with monotherapy. We previously reported that serum and urine IL-18 concentrations are predictors of diabetic nephropathy²¹. IL-18 secretion in monocytes and macrophages is stimulated by inflammation and oxidative

stress associated with hyperglycemia²⁵. These results suggest that the combination of losartan and imidapril might be useful to prevent the development of early nephropathy in patients with type 2 diabetes by decreasing ACR, and reducing inflammation and oxidative stress in the kidney through more potent inhibition of the RAS.

The main limitation of the present study was that the observation period was not long enough to evaluate renal or cardiovascular outcomes. The Olmesartan Reducing Incidence of Endstage Renal Disease in Diabetic Nephropathy Trial (ORIENT)²⁶, and Ongoing Telmisartan Alone and in Combination with Ramipril Global End Point Trial (ONTARGET)²⁷ studies both showed that combination therapy with an ACEI and an ARB did not have significant benefits on renal outcomes. However, in the ORIENT study²⁶, combination therapy with olmesartan and an ACEI reduced proteinuria, as observed in the present study, but did not further improve renal or cardiovascular outcomes. The reason for these discrepancies remains unclear. Further studies are required to confirm the beneficial effect of combination therapy with an ARB and an ACEI on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy.

In the present study, the sample size of 28 patients was small, meaning larger studies are required to confirm these results. Although the serum and urinary concentrations of several biomarkers decreased after monotherapy or combination therapy, some changes were not statistically significant, perhaps because of the small sample size. Although amlodipine was used during the washout period to prevent possible changes in BP, we measured ambulatory BP at a single time, so we cannot exclude the possible effects of BP on the decrease in ACR. A larger clinical study using 24-h BP monitoring might be required to verify the results of the present study.

In conclusion, a combination of an ACEI and an ARB could be beneficial for treating diabetic nephropathy through their anti-inflammatory and anti-oxidative stress effects.

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Pathology of glomerular lipidosis

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Abstract Glomerular lipid deposition is sometimes associated with a particular kind of lipid metabolism disturbance. Ultrastructural analyses using electron microscopy often indicate a disease-specific aspect of intraglomerular lipid distribution.

Keywords Glomerular lipidosis · Foam cell · Lipoprotein glomerulopathy · LCAT deficiency · Fabry disease

Introduction

Lipid deposition is frequently observed in kidney biopsy specimens. Here, we focus attention on glomerular lipidosis in which there are several glomerular disorders associated with specialized lipid metabolism disturbance. Electron microscopic examination provides a number of interesting findings on the morphological character of lipid depositions. According to the ultrastructural localization, intraglomerular lipid distribution may be roughly classified into the following four categories—lipid accumulation in the infiltrated macrophages, in the glomerular capillary

lumens without foam cells, in the mesangial and/or sub-endothelial area, and in the glomerular epithelial cells.

Lipid accumulation in the infiltrated macrophages

This type of glomerular lipidosis is usually recognized as intracapillary foam cells (Fig. 1), and is typically observed in the early stage of focal segmental glomerulosclerosis [1] or diabetic glomerulosclerosis [2], and in familial type III hyperlipoproteinemia [3]. Similar findings are also found in various glomerular diseases with no particular specificity.

Lipid accumulation within the glomerular capillary lumens without foam cells

This form of glomerular lipidosis is typically seen in lipoprotein glomerulopathy [4]. In this disease, enlargement of glomerular capillary lumens is the most outstanding observation (Fig. 2a). Although each capillary lumen only looks like a vacuum space without any solid substances on light microscopy, electron microscopy reveals that the largely expanded lumens are filled with numerous cobblestone-like materials with no cellular components, like intracapillary lipid thrombi (Fig. 2b).

Lipid accumulation in the mesangial and/or subendothelial area

This type was representatively demonstrated in a patient in our institute with lecithin-cholesterol acyltransferase (LCAT) deficiency [5]. Electron microscopy revealed numerous lipid particles in the mesangial and subendothelial

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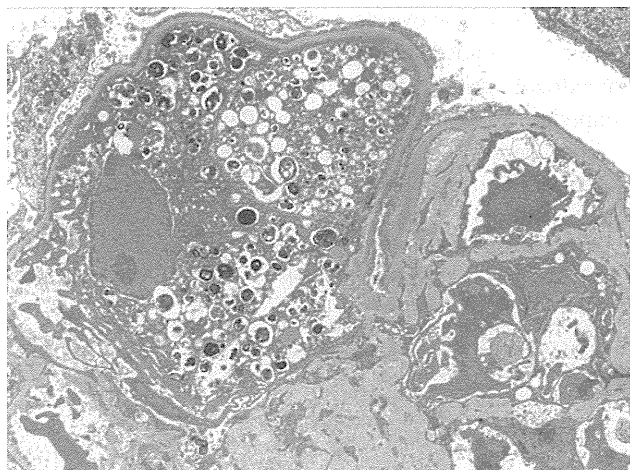


Fig. 1 Intracapillary foam cells in a patient with an early stage of focal segmental glomerulosclerosis. Electron microscopy, $\times 5,000$

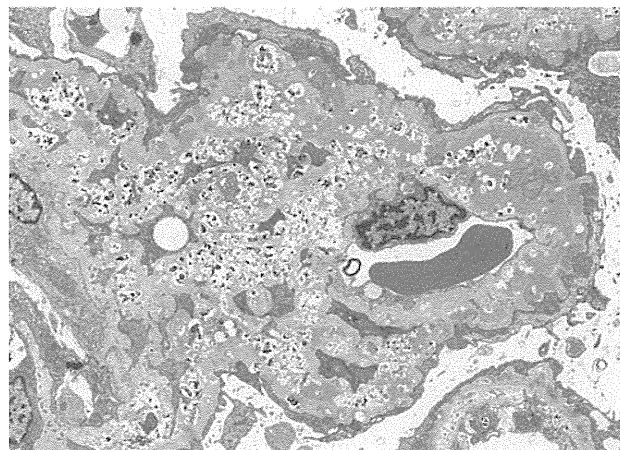


Fig. 3 Numerous lipid particles accumulated in mesangial and subendothelial space in a patient with LCAT deficiency. A number of subepithelial electron-dense deposits and spikes are also observed outside the glomerular basement membrane. Electron microscopy, $\times 4,000$

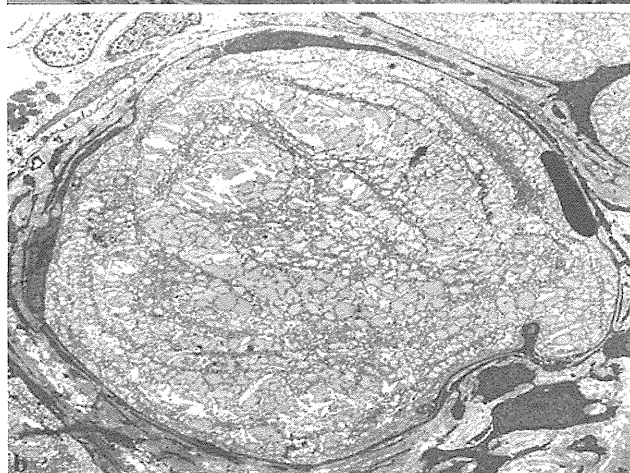
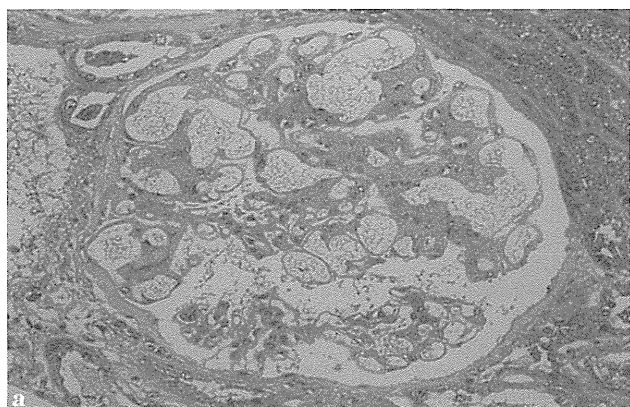


Fig. 2 a Marked enlargement of glomerular capillary lumens in a patient with lipoprotein glomerulopathy. Elastica-Masson stain, $\times 400$. **b** Electron microscopy reveals numerous cobblestone-like materials fills the glomerular capillary lumen, $\times 4000$

space. A number of subepithelial electron-dense deposits and spikes were also observed outside the glomerular basement membrane, indicating a complication of stage II membranous nephropathy (Fig. 3).

Interestingly, this case showed only a clinical manifestation of LCAT deficiency without any genetic abnormalities specific to LCAT deficiency. Corticosteroid treatment improved not only the kidney-related manifestation including proteinuria but also all of the clinical phenomena associated with LCAT deficiency. It is speculated that some kind of immune-mediated non-genetic mechanisms played a key role on the impaired activity of LCAT although the precise mechanism has not yet been clarified. Takahashi et al. [6] recently reported a similar patient with acquired LCAT deficiency in which membranous nephropathy was complicated in the same way as in our case. They demonstrated the presence of an inhibitory anti-LCAT antibody in the patient's serum, and immunohistochemistry detected LCAT along parts of the glomerular capillary walls, suggesting that LCAT was the antigen responsible for the membranous nephropathy.

Lipid accumulation in the glomerular epithelial cells (Fig 4)

Fabry disease reveals a characteristic vacuolar degeneration of the glomerular epithelial cells deriving from an excessive deposition of neutral globotriaocylceramide (GL3).

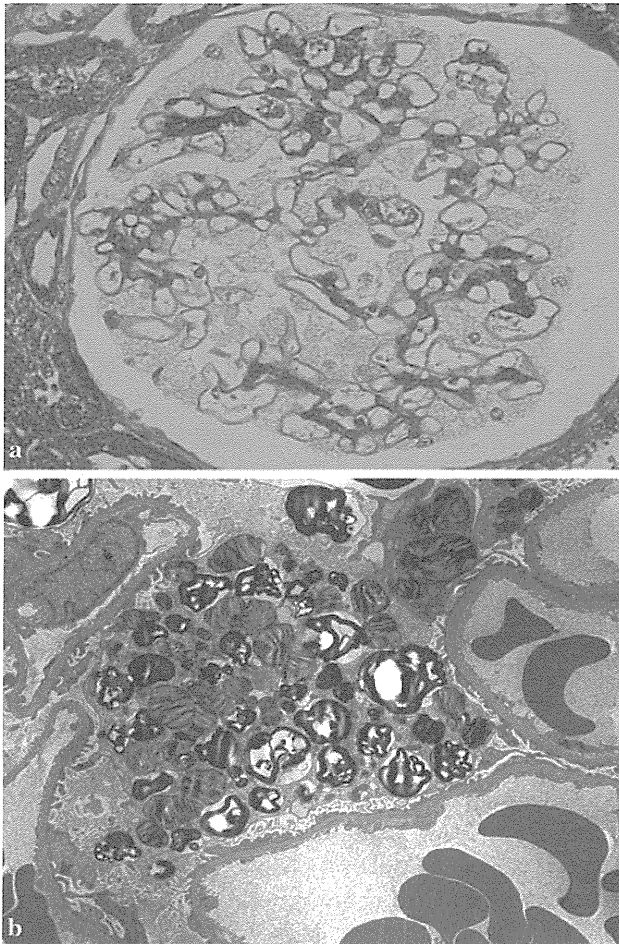


Fig. 4 **a** Bubbling appearance of the glomerular epithelial cells in a patient with Fabry disease. Azan-Mallory stain, $\times 400$. **b** So-called zebra-bodies are recognized on electron microscopy, $\times 4,000$

Although the above-described histopathological classification of glomerular lipidosis may contain only a few exceptional cases or borderline problems, an unequivocal comprehension of the intraglomerular distribution pattern of lipid deposition undoubtedly contributes to adequate understanding of lipid-associated kidney injuries.

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Conflict of interest None.

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The Japanese version of the modified ACR Preliminary Diagnostic Criteria for Fibromyalgia and the Fibromyalgia Symptom Scale: reliability and validity

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Abstract

Purpose The aim of this study is to investigate the reliability and validity of the Japanese version of the modified American College of Rheumatology (ACR) Preliminary Diagnostic Criteria for Fibromyalgia (mACR 2010-J) and the Fibromyalgia Symptom Scale (mFS-J).

Methods According to the ACR 1990 classification criteria, patients with chronic pain were divided into the fibromyalgia group and nonfibromyalgia group (rheumatoid arthritis and osteoarthritis). Patients in both groups were assessed using mACR 2010-J and mFS-J.

Results 294 of 462 (64 %) patients in the fibromyalgia group met mACR 2010-J, whereas 4 % (9/231) of the nonfibromyalgia group did, with sensitivity of 64 %, specificity of 96 %, positive predictive value of 97 %, negative predictive value of 56 %, and positive likelihood ratio of 16.3. Mean total scores on mFS-J significantly differentiated the fibromyalgia from the nonfibromyalgia

group. According to the value of the Youden index, the best cutoff score for the mFS-J was 9/10.

Conclusion Our findings indicate that mACR 2010-J as a positive test and mFS-J as a quantification scale might be suitable for assessing fibromyalgia among Japanese chronic pain populations.

Keywords Diagnostic criteria · Fibromyalgia · Symptom scale · Modified ACR Preliminary Diagnostic Criteria for Fibromyalgia

Introduction

Fibromyalgia (FM) is characterized by widespread musculoskeletal chronic pain, fatigue, poor sleep, frequent psychological difficulties, and multiple tender points on physical examination [1, 2]. In 1990, the American College

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of Rheumatology (ACR) presented FM criteria (ACR 1990) that required tenderness on pressure (tender points) in at least 11 of 18 specified sites and the presence of widespread pain for diagnosis [1]. Widespread pain was defined as axial pain, both left- and right-sided and with upper and lower segment pain. However, ACR 1990 had the serious problem of little variation in symptoms. To improve this shortcoming, new clinical criteria, which integrate variations in symptoms with severity scale (2010 ACR Preliminary Diagnostic Criteria for FM, ACR 2010) [3], have been presented. The diagnostic criteria for FM are satisfied if the following three conditions are met: (1) Widespread Pain Index (WPI) ≥ 7 and Symptom Severity Score (SS) ≥ 5 , or WPI of 3–6 and SS ≥ 9 ; (2) symptoms have been present at a similar level for at least 3 months; and (3) the patient does not have a disorder that would otherwise explain the pain. The publication of ACR 2010 eliminated the tender point examination, thus making it possible to study FM in survey and clinical research.

Accordingly, we have validated the Japanese version of ACR 2010 [4]. In addition, we have originally validated the Japanese version of the Fibromyalgia Symptom Scale with the sum of WPI and the original SS, i.e., fatigue, waking unrefreshed, cognitive symptoms, and somatic symptoms in general consisting of 41 symptoms of the FS-J [4]. Both ACR 2010-J and FS-J have high reliability and validity, and are useful for assessing fibromyalgia among Japanese chronic pain populations.

Recently, Wolfe et al. [5] proposed a modification of the ACR 2010 (mACR 2010), deleting 38 out of 41 somatic symptoms in general from the original SS. Consequently, complete self-administration has become possible. Furthermore, they created the Fibromyalgia Symptom Scale with the sum of WPI and the new SS (FS). They reported that the criteria properly identified diagnostic groups, and that FS score ≥ 13 best separated criteria+ and criteria- patients.

The aim of this study is to investigate the reliability and validity of the Japanese version of the mACR 2010 (mACR 2010-J) and the Japanese version of the FS (mFS-J). Furthermore, our questions are whether mACR 2010-J would be more useful than ACR 2010-J for assessing fibromyalgia among Japanese chronic pain populations, and whether mFS-J is more suitable than FS-J as a positive test.

Subjects and methods

An experienced rheumatologist and an experienced psychiatrist had translated the mACR 2010 into Japanese with the author's permission and produced forward- and back-translations to create the mACR 2010-J.

We recruited FM patients who met the previous criteria of the ACR 1990 and were without psychiatric disorders

according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [6] in a clinic specialized for FM, the Kasumigaseki Urban Clinic, in Tokyo, Japan, between August 1, 2010 and July 31, 2011. During the study period, other patients with diseases associated with chronic pain such as rheumatoid arthritis (RA) and osteoarthritis (OA) who had not been diagnosed previously with FM were recruited as control patients. To adjust the imbalance of number of patients, control patients were additionally recruited from May 30 to July 2, 2012. The diagnoses of RA and OA were made according to the 2010 rheumatoid arthritis classification criteria [7] and the American College of Rheumatology criteria for classification and reporting of osteoarthritis of the hand, hip, and knee [8–10]. The experienced rheumatologist and the experienced psychiatrist familiar with FM assessed these patients. This study was approved by the Institutional Review Board of Kasumigaseki Urban Clinic.

After obtaining informed consent from study participants, the rheumatologist rated patients with the mACR 2010-J. In order to assess interrater reliability, another rater independently rated a subset of the same subjects ($N = 19$) while blind to the diagnoses and scores of the other rater. The raters in this study were already fully trained in use of the scale and quite experienced in use of it. We therefore decided that only a small subsample was needed to reevaluate consistency across raters.

Statistics

Data were analyzed using SPSS 17.0-J software. Differences among groups in demographic and clinical characteristics were calculated with the unpaired *t* test. If data were not sampled from Gaussian distributions, a nonparametric test (Mann–Whitney *U* test) was used. To compare categorical data, we used Fisher's exact test.

In the present study, the control group was not healthy volunteer but consisted of chronic pain patients with RA and OA. It has been reported that the age-specific incidence of RA peaked in the 60–64 and 70–74 year age groups for females and males, respectively, in Taiwan [11]. Similarly, it has been reported that the peak prevalence of knee OA in women and men was ≥ 80 years in Japan [12]. In contrast, we have reported that the frequent age of onset of FM in women was 35–55 years based on our FM database including 3,500 Japanese patients with FM [13]. Among Asians, thus, patients with FM are much younger than those with RA and OA. Therefore, matching age of control patients with age of FM patients seems to be rather arbitrary. Accordingly, to control for the effect of age on the rate of patients meeting the mACR 2010-J, patients were divided into three age categories, i.e., 20–39, 40–59, and ≥ 60 years.

There were only eight FM patients and one non-FM patients less than 20 years of age, and there were only two FM patients and three non-FM patients 80 years or older. Then, the Mantel–Haenszel method was used to test the difference in the percentage of patients meeting the mACR 2010-J between the two groups. Also, to control for the effect of age on the score on the mFM-J, one-way analysis of covariance was used. The internal consistency for the mFM-J was calculated with Cronbach's α . Interrater reliability was measured with the intraclass correlation coefficient (ICC) for pairs of independent raters. Cutoff scores for the mFS-J were determined using receiver-operator characteristic (ROC) analyses to determine the Youden index when comparing the FM group with all non-FM subjects. Positive predictive value (PPV), negative predictive value (NPV), and positive likelihood ratio (sensitivity/1 – specificity) were also calculated. All statistical tests were two-tailed. Statistical significance was set at $p < 0.05$.

Results

A total of 462 patients meeting the ACR 1990 (the FM group) and a total of 231 non-FM patients (RA patients,

196; OA patients, 35; the non-FM group) were enrolled. Demographic and clinical characteristics of the groups are presented in Table 1, showing that 294 of 462 (64 %) patients in the FM group met the mACR 2010-J, whereas 4 % (9/231) of the non-FM group did, including 4 % (8/196) of RA patients and 3 % (1/35) of OA patients. The percentage of patients meeting the mACR 2010-J criteria in the FM group was significantly higher than that of the non-FM group after adjusting for age (estimated odds ratio, 35.7, $p < 0.0001$; Table 1). The sensitivity, specificity, PPV, NPV, and positive likelihood ratio for comparison of the FM group with all non-FM subjects were 64, 96, 97, 56, and 16.3 %, respectively. The ICC between the two independent raters was very high for the mACR 2010-J, at 0.877.

The mean score (standard deviation, SD) of mFS-J in the FM group was 16.7 (6.5), while that in the non-FM group was 3.7 (4.1). The mean score of mFS-J in the FM group was significantly higher than that of the non-FM group after adjusting for age ($F = 605.1$, $p < 0.0001$; Table 1). Internal consistency was not high, with a Cronbach's α coefficient for the mFS-J (WPI + the modified SS) of 0.603. ROC analyses were performed for the mFS-J, comparing the FM group with the non-FM group. Table 2

Table 1 Demographic and clinical characteristics of the fibromyalgia group and nonfibromyalgia group

Group	Fibromyalgia ($N = 462$)	Nonfibromyalgia ($N = 231$)		p
		RA ($N = 196$)	OA ($N = 35$)	
Mean age (SD), years	50.6 (14.8)	61.3 (13.9)		<0.0001
		60.6 (14.4)	65.5 (10.2)	
Sex (female), N (%)	389 (84)	188 (81)		0.39
		160 (82)	28 (80)	
Patients meeting the mACR 2010-J, ^a N (%)	294 (64)	9 (4)		<0.0001 ^c
		8 (4)	1 (3)	
Mean score (SD) of mFS-J ^b	16.7 (6.5)	3.7 (4.1)		<0.0001 ^d
		3.7 (4.2)	3.9 (3.3)	

^a The Japanese version of the modified 2010 ACR Preliminary Diagnostic Criteria for Fibromyalgia

^b The Japanese version of the Fibromyalgia Symptom Scale (WPI + modified SS)

^c To control for the effect of age on the rate of patients meeting the mACR 2010-J, patients were divided into three age categories. Then, the Mantel–Haenszel method was used to test the difference in the percentage of patients meeting the mACR 2010-J between the two groups

^d To control for the effect of age on the score of the mFM-J, one-way analysis of covariance was used

SD standard deviation

Table 2 Sensitivity and specificity of the Japanese version of the Fibromyalgia Symptom Scale (mFS-J), based on receiver-operating characteristics (ROC) analysis: fibromyalgia group versus nonfibromyalgia (RA and OA) group

Cutoff score	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Youden index
8.5	87.7	89.2	8.1	0.769
9.5	84.8	92.2	10.9	0.770
10.5	82.0	92.2	10.5	0.742

shows the sensitivity, specificity, positive likelihood ratio, and Youden index for ROC analysis at various cutoff scores for the mFS-J. According to the value of the Youden index, the best cutoff score for the mFS-J was 9/10.

Discussion

This is the first study to validate the mACR 2010-J and mFS-J, which is the quantification scale of the mACR 2010-J. The positive likelihood ratio of 16.3 for the mACR 2010-J is sufficiently high as a positive test. We cannot directly compare the likelihood ratios for the mACR 2010-J and the ACR 2010-J, as the present study group is quite different from that used in the study of the ACR 2010-J [4]. However, the value of mACR 2010-J is sufficiently high compared with that of ACR 2010-J, for which the positive likelihood ratio was 8.8. Therefore, the modification of ACR 2010-J may be superior to the original ACR 2010-J as a positive test.

The best cutoff score for the mFS-J was 10, which is just the same as the FS-J [4]. Furthermore, the positive likelihood ratio for the mFS-J (Table 2) is as high as that for the FS-J at the cutoff score [4]. As the mFS-J is simpler than the FS-J, mFS-J may be superior to FS-J based on the original ACR 2010-J as a quantification scale. Meanwhile, the best cutoff score of 10 in the present study is smaller than that of the original study on the FS performed in the USA (cutoff score 13) [5]. One explanation for this difference is in patient characteristics. In the present study, comorbid psychiatric disorders were excluded, while in the previous study they were not. Patients with major depressive disorder, panic disorder, or anxiety disorder usually have somatic symptoms similar to those of ACR 2010, and comorbidity of major depressive disorder, panic disorder, or anxiety disorder is not rare [1]. Therefore, the population in the previous study may have been modified by comorbid psychiatric disorders. Thus, the cutoff score of 10 in the present study might reflect fibromyalgia itself more than that of 13 in the previous study. Another possible explanation is cross-cultural differences in expression or rating of symptoms.

The internal consistency with a Cronbach's α coefficient for the mFS-J (WPI + the modified SS) of 0.603 is lower than that for the FS-J (WPI + the original SS) of 0.747 [4]. The modified SS consists of fatigue, waking unrefreshed, cognitive symptoms, plus having pain/cramps in the abdomen, depression, and headache, resulting from 38 somatic symptoms in general having been deleted from the original SS. Therefore, the modified SS values neuropsychiatric symptoms more than the original SS. In contrast, WPI is the number of pain areas, which is simply somatic. Accordingly, the internal consistency for the mFS-J (WPI + the modified SS) might have been lower than that

for the FS-J (WPI + the original SS). As FM shows a variety of symptoms and is suspected of involving not only musculoskeletal but also central nervous system [14], the not so high value of internal consistency for the mFS-J may not necessarily be a shortcoming.

Thus, mACR 2010-J as a positive test and mFS-J as a quantification scale might be suitable for assessing fibromyalgia among Japanese chronic pain populations. A strength of the present study is that the findings represent real clinical practice in Japan, since the study was performed in a clinic specialized in FM which is visited by the largest number of FM patients in Japan. A limitation of this study is that the findings may not be applicable to all patients, since FM patients with other musculoskeletal diseases, such as spondylitis, were not included in it. Further studies with patients of other countries or ethnicities will be needed to determine cross-cultural or ethnic differences in expression or rating of symptoms.

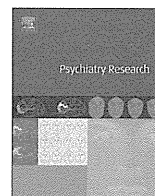
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Conflict of interest None.

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The possibility that requiring high-dose olanzapine cannot be explained by pharmacokinetics in the treatment of acute-phase schizophrenia



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ABSTRACT

We examined clinical characteristics including serum olanzapine concentrations for acute schizophrenia patients who required above conventional doses. We performed a rater-blinded, randomized clinical trial in 12 psychiatric emergency sites. Eligible patients were 18–64 years old and met diagnostic criteria for schizophrenia, acute schizophrenia-like psychotic disorder, or schizoaffective disorder. A total of 42 patients were randomly assigned by means of sealed envelopes to receive risperidone (3–12 mg/day; $n=20$) and olanzapine (10–40 mg/day; $n=22$), with follow-up at 8 weeks. The Negative score of the Positive and Negative Syndrome Scale was significantly higher in patients who required high doses than in patients who responded to conventional doses. Serum olanzapine concentrations at the time of oral 20 mg/day could be obtained from 5 out of 7 patients who subsequently required high-dose olanzapine. All values were more than 30 ng/mL after 11–16 h from dosing to sample collection, and the mean value was 47.876 (S.D. 21.546) ng/mL. Such concentrations are appropriate with respect to a therapeutic range of 20–50 ng/mL. The present study has shown evidence that the reason for requiring high-dose olanzapine cannot be explained by pharmacokinetics in the treatment of acute-phase schizophrenia.

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1. Introduction

Olanzapine and risperidone are most frequently used among second generation antipsychotics for the acute treatment of psychosis

in hospitalized patients (Choi et al., 2011), as the superiority of them has been reported in several randomized clinical trials (RCTs) (Kraus et al., 2005; McCue et al., 2006; Hatta et al., 2009). However, no difference in effectiveness between the two antipsychotics has been reported in the previous RCTs, in which both antipsychotics were given within the licensed doses, i.e. 20 mg/day for olanzapine and 12 mg/day for risperidone. Remarkably, the upper dose of olanzapine is equivalent to half of the upper dose of risperidone (Gardner et al., 2010). Therefore, a clinical question has been raised whether

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olanzapine would be superior to risperidone when olanzapine is allowed to be given above the licensed dose as needed.

In clinical practice, it has been reported that nearly 50% of olanzapine prescription were above 20 mg/day in the U.S. (Citrome et al., 2007) and that the median for olanzapine recommendation dose by U.S. experts was 30 mg/day (Gardner et al., 2010). The upper limit of olanzapine dose in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study, in which olanzapine was the most effective in terms of the rates of discontinuation, was designed to be 30 mg/day (Lieberman et al., 2005).

In chronic schizophrenia inpatients that showed suboptimal response to treatment, there is a RCT that the use of high doses of olanzapine were allowed (Volavka et al., 2002). In the study, it has been reported that clozapine and olanzapine were superior to haloperidol, but that the superiority of risperidone over haloperidol was not obvious. In treatment-resistant schizophrenia patients, there were reports that olanzapine at higher than customary doses demonstrated similar efficacy to clozapine (Meltzer et al., 2008) or less effective than clozapine (Kumra et al., 2008). Meanwhile, such a RCT has not been conducted in acute-phase schizophrenia patients.

Another clinical question was who needs high-dose risperidone or olanzapine. Especially, olanzapine has little active metabolites (Callaghan et al., 1999), in contrast to risperidone. Furthermore, there is a high correlation between serum and cerebrospinal fluid olanzapine concentrations (Skogh et al., 2011). Therefore, serum olanzapine concentrations would reflect most activity of olanzapine. Accordingly, serum olanzapine concentrations for patients who require high doses are worth measuring to investigate pharmacokinetic characteristics of such patients. However, serum olanzapine concentrations at 20 mg/day for patients who do not respond to conventional doses have not been well investigated.

We therefore prospectively examined whether olanzapine within 40 mg/day would be superior to risperidone within 12 mg/day in acute schizophrenia patients. In addition, we examined clinical characteristics for patients who required above conventional doses. Especially, in order to investigate whether serum olanzapine concentrations for patients who did not respond to conventional doses would be inappropriately low, serum olanzapine concentrations were measured. The present study was performed with emergency-based, newly admitted patients without support from pharmaceutical companies, reflecting real-world practice.

2. Methods

2.1. Setting and participants

Of the 80 psychiatric emergency wards authorized by the Japanese government, 12 (15%) participated in the present study. These wards were located all over Japan, and were responsible for local emergency cases. Most admissions to these hospitals represented behavioral emergencies and approximately 60% were brought in by the police. All were involuntary admissions as an immediate danger to themselves or others, according to the 1995 Law Concerning Mental Health and Welfare for the Mentally Disabled. Details of the clinical setting are described elsewhere (Hatta et al., 1998). According to government policies, psychiatric emergency services have been expanded in both metropolitan and local areas over the last 17 years. The quality of sites and patients in the present study was therefore homogenous. This activity was conducted by the Japan Acute-phase Schizophrenia Trial (JAST) study group (Hatta et al., 2009, 2011, 2012).

During the study period, between June 1, 2011 and January 31, 2012, a total of 1746 patients were admitted and assessed for eligibility. Eligible patients were 18–64 years old, newly admitted as emergency cases, and meeting the criteria of the DSM-IV-TR for schizophrenia, schizophreniform disorder, or schizoaffective disorder. Patients with obvious complications such as liver dysfunction, renal dysfunction, heart failure, respiratory failure, or diabetes mellitus were excluded, as were patients who were pregnant or who wanted to become pregnant.

2.2. Study design

All study protocols were approved by the institutional review board at each site, and written informed consent was obtained from patients or their legally

authorized representatives. Patients who refused oral medication were initially treated with injections. Times of injections before enrollment were not limited. After resolution of agitation, the investigators informed patients orally and in writing about the trial, and invited them to participate.

Patients were randomized using the sealed envelope method in a rater-blind manner to either risperidone or olanzapine for 8 weeks. For randomization, we referred to a random number table, with sequentially numbered, opaque, sealed envelopes used to conceal the allocation sequence.

The initial doses of risperidone and olanzapine were 3 mg/day and 10 mg/day, respectively. Doses were subsequently increased or decreased at the discretion of the treating psychiatrist. The maximum of licensed dose for olanzapine is 20 mg/day, which is equivalent to 6 mg/day for risperidone (Gardner et al., 2010). Therefore, the definition of higher dose in the present study was more than 20 mg/day for olanzapine and more than 6 mg/day for risperidone. The maximum doses of risperidone and olanzapine were 12 mg/day and 40 mg/day, respectively, considering dose equivalency (Gardner et al., 2010). Use of benzodiazepines was allowed and documented. Use of valproate as a mood stabilizer was also allowed and documented. However, use of other mood stabilizers and antidepressants was not permitted. Use of anticholinergic drugs was also not allowed unless acute extrapyramidal side effects appeared.

2.3. Procedures

Before starting the trial, site-coordinators were trained to assess outcomes as raters. All site-coordinators were experienced psychiatrists. A training video was used to train raters in assessment of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1991). The primary outcome measure was all-cause discontinuation by 8 weeks.

Efficacy outcomes consisted of PANSS, the Clinical Global Impressions-Improvement Scale (CGI-I: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; and 7, very much worse) (Guy, 1976), and the Global Assessment of Functioning (GAF) (Jones et al., 1995). Safety and tolerability outcomes were determined based on vital signs, weight, laboratory data, electrocardiography (ECG), and the Drug-induced Extrapyramidal Symptom Scale (DIEPSS), which includes parkinsonism, akathisia, dystonia, and dyskinesia (Inada, 1996). Data including PANSS, CGI, GAF, vital signs, weight, laboratory data, ECG, and DIEPSS were collected on admission and every 2 weeks thereafter. Data were also collected at the time of discontinuation of the allocated treatment. Sexual side effects were recorded when reported by patients, and sedation was recorded when described by patients as an aversive subjective experience or when observed. Raters did not work on the wards involved in the study, were not involved with treatment, and were blinded to the drug assignments. The tested drug was discontinued when the treating psychiatrist judged the efficacy of the drug to be insufficient, when the treating psychiatrist judged side-effects of the drug to be intolerable, or when the patient reported non-adherence. Before a judgment of insufficient efficacy could be made, the drug dosage was increased to the maximum. The definition of drug non-adherence in the study performed in emergency and acute wards, where drug administration was managed by nurses, was that a patient declines the continuation of assigned medication.

2.4. Determination of olanzapine in human serum

Fasting blood samples were collected in the morning at least 10 h after the last evening dose of olanzapine for analyses of olanzapine. Serum for concentration analysis was separated and stored frozen at -20°C until analysis.

A liquid chromatography/tandem mass spectrometry method was used for analysis of olanzapine. Olanzapine and internal standard (IS: LY170222) were extracted from serum (100 μL) by liquid-liquid extraction using tert-butyl methyl ether. The processed sample (10 μL) was injected into high-performance liquid chromatography/tandem mass spectrometer that was equipped with a C_{18} column, using 10 mmol/L ammonium acetate/acetonitrile and acetonitrile as the mobile phase under gradient conditions. Olanzapine and IS were detected using a multiple reaction monitoring mode with positive ion (olanzapine: m/z 313 \rightarrow 256; IS: m/z 327 \rightarrow 270). Calibration for olanzapine was linear within the concentration range of 0.25–100 ng/mL.

2.5. Statistical analysis

Differences between categorical variables in patient demographics and clinical characteristics were calculated using Fisher's exact test. Differences between sequential variables were calculated using the unpaired t test (with Welch correction if applicable). If data were not sampled from Gaussian distributions, a non-parametric test (Mann-Whitney test) was used. Mean improvement in the PANSS total score was calculated as $100 \times (\text{baseline score} - \text{week} \times \text{score}) / (\text{baseline score} - 30)$ (Leucht et al., 2009). Kaplan-Meier curves were used to estimate the probability of treatment discontinuation at 8 weeks. Statistical analyses were performed using SPSS version 17.0J software (SPSS, Tokyo, Japan). All statistical tests were two-tailed. Values of $P < 0.05$ were regarded as statistically significant.

In our previous randomized clinical study, 25% of patients allocated to risperidone, of which the maximum dose was allowed up to 12 mg/day, discontinued risperidone by 8 weeks, while 12% of patients allocated to olanzapine, of which the maximum dose was allowed up to 20 mg/day, discontinued olanzapine by 8 weeks (Hatta et al., 2009). In the present study, the maximum dose of olanzapine was allowed up to 40 mg/day. As no previous data are available regarding the discontinuation rate for patients allocated to olanzapine with such conditions, we assumed 10% decrease in discontinuation rate by 8 weeks from our clinical experiences. The statistical power was set as $\text{power}=1-\beta=80\%$, and sensitivity as $\alpha=5\%$ to enable detection of differences in the effects of the augmentation strategy. Power analysis consequently set the required number of patients at 34 patients per group.

This study is registered in the UMIN Clinical Trials Registry (number: UMIN000005526; <http://www.umin.ac.jp/ctr>).

3. Results

3.1. Comparison between patients allocated to risperidone and patients allocated to olanzapine

Fig. 1 shows the trial profile. Forty-two patients were randomly assigned to two treatment groups. Baseline characteristics of randomized patients were much the same between groups, and mean (\pm S.D.) maximum doses of risperidone and olanzapine were 6.9 ± 2.7 mg/day and 23.0 ± 10.2 mg/day, respectively, suggesting relative dose equivalency. However, the number of patients allocated to each treatment group did not reach the required number of patients set by power analysis. Therefore, it is not conclusive about the primary outcome measure although time to treatment discontinuation for any cause did not differ between treatment groups (47.0 days [95%CI 39.9–54.0] for risperidone vs. 47.0 days [40.0–54.0] for olanzapine, $P=0.93$).

With respect to safety and tolerability outcomes, the rate of extrapyramidal symptoms was significantly higher in patients taking risperidone than in patients taking olanzapine ($P=0.0080$), corresponding to the significant difference in the rate of adjunctive anticholinergic drug use between the groups ($P=0.013$). No significant differences between treatment groups were identified in mean change from baseline for fasting glucose, cholesterol, triglycerides, or weight. Over-sedation was observed in one patient taking

olanzapine (max. dose, 30 mg/day). Sexual side effects were not observed.

No significant difference in the rate of patients who required high doses was seen between the risperidone group and the olanzapine group (40% [8/20] vs. 32% [7/22], $P=0.75$). The rates of patients who achieved a $\geq 50\%$ improvement in PANSS total score by 8 weeks in patients requiring high-dose risperidone and in patients requiring high-dose olanzapine were 25% [2/8] and 0% [0/7], respectively. Meanwhile, the rates of patients who achieved moderate ($\geq 30\%$) improvement in PANSS total score in patients requiring high-dose risperidone and in patients requiring high-dose olanzapine were 63% [5/8] and 57% [4/7], respectively.

3.2. Comparison between patients having required high doses and patients having responded to conventional doses

Fifteen patients required high doses. Of these patients, six patients were drug-naïve. Among the rest nine patients, only one patient that was allocated to risperidone met the definition of treatment-resistant schizophrenia at the time of study entry (Suzuki et al., 2011). The high-dose group was in a greater trend in the mean PANSS total score at baseline than the conventional-dose group ($P=0.051$, Table 1). In line with it, the high-dose group was in a greater trend in the rate of patients who received haloperidol injections at the time of admission than the conventional-dose group ($P=0.085$). Also, the high-dose group was in a greater trend in the times of haloperidol injections at the time of admission than the conventional-dose group (median 1 vs. 0, $P=0.098$). All subscale scores of PANSS were very high in both groups. Although there were no significant differences in scores of PANSS Positive scale and General psychopathology scale between groups, PANSS Negative scale score was significantly higher in the high-dose group than in the conventional-dose group ($P=0.0077$).

The mean PANSS total score at the time of starting high doses in the high-dose group was 104.5 (S.D. 21.5), which is as high as that at baseline in the conventional-dose group (105.2 [S.D. 24.8], Table 1).

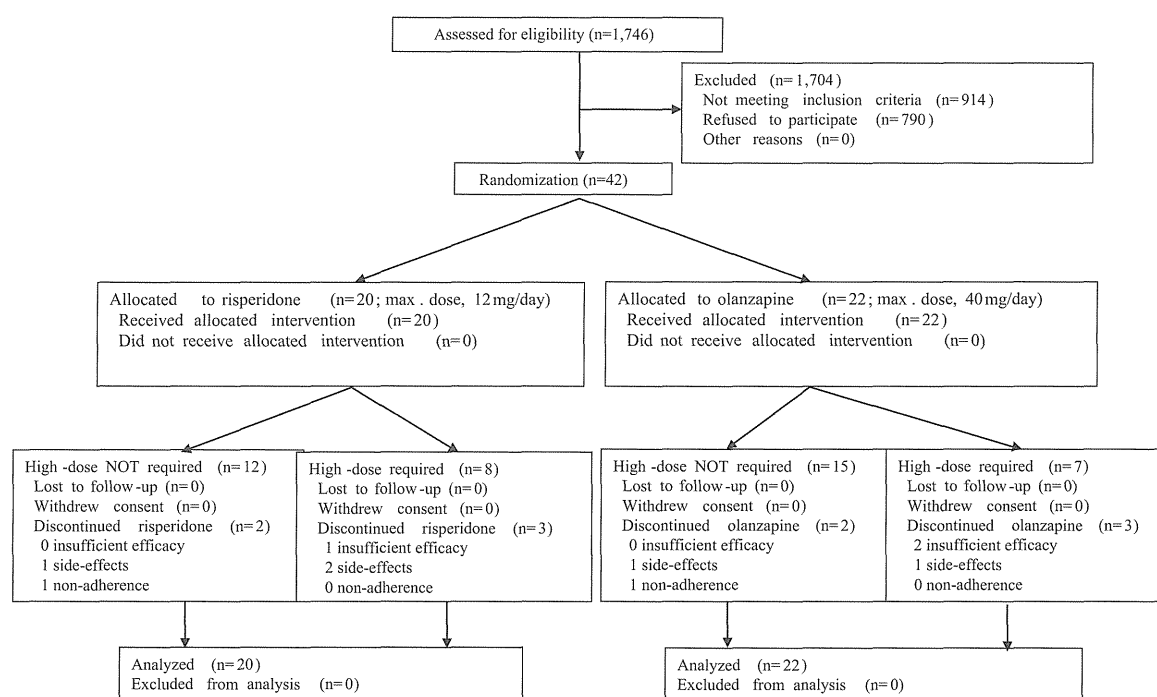


Fig. 1. Trial profile.