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Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia

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Abstract

Nonalcoholic steatohepatitis (NASH) is the hepatic manifestation of the metabolic syndrome. Currently, there is no established therapy for NASH. The aim of the present study was to evaluate the efficacy of atorvastatin in the treatment of NASH associated with hyperlipidemia. This prospective study included 31 patients with biopsy-proven NASH with hyperlipidemia. Body mass index, serum lipids, liver function tests, fibrosis markers, and adipocytokines (adiponectin, leptin, tumor necrosis factor–α) were measured periodically during an open-label study of atorvastatin (10 mg daily) for 24 months. Standard weight-loss counseling was continued during the treatment period. Oral glucose tolerance test and liver density assessed by computerized tomography were performed before and after treatment. Follow-up liver biopsy was performed in 17 patients. All 31 patients had high cholesterol levels at baseline, and 20 also presented high triglyceride levels. The body mass index and serum glucose levels did not change during the treatment. After treatment, 23 patients (74.2%) presented normal transaminase levels. Adiponectin levels were significantly increased, and the levels of tumor necrosis factor–α were significantly decreased. However, leptin levels were not changed significantly. The concentration of long-chain fatty acids was decreased; and significant decreases were observed in C18:2,n-6 (linoleic acid, −21%) and C20:4,n-6 (arachidonic acid, −22%). Liver steatosis and nonalcoholic fatty liver disease activity score were significantly improved, whereas 4 patients had increased fibrosis stage. The NASH-related metabolic parameters improved with therapy, including fibrosis in some patients. However, 4 of 17 patients had progression of fibrosis over the 2-year period, with 3 of them progressing to stage 3. It is unclear whether this divergent response represents sampling error, heterogeneity in the population, or untreated postprandial hyperglyceridemia. Controlled trials are needed to further investigate and resolve this.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). Whereas simple steatosis seems to be a benign and nonprogressive condition, NASH is recognized as a potentially progressive disease that can lead to cirrhosis, liver failure, and hepatocellular carcinoma [1-4]. In Western countries, the prevalence in the general population of NASH ranges from 1% to 5%; and that of NAFLD ranges from 15% to 39% [5,6]. In Japan, a quarter of Japanese adults have become overweight, approximately 20% of Japanese adults have NAFLD, and about 1% of those are estimated to be have NASH as well [7,8]. Thus, the prevalence of NAFLD and NASH is increasing and becoming a major target disease not only in Western countries but also in Japan.

Nonalcoholic steatohepatitis is considered the hepatic manifestation of the metabolic syndrome and is particularly associated with insulin resistance (IR), obesity, hypertension, and abnormalities in glucose and lipid metabolism [9-12]. Currently, there are no proven effective therapies available for the treatment of NASH; and strategies have mainly led to treat underlying risk factors [13,14]. Promising treatments for NASH include antioxidants, hepatoprotective agents, antidiabetic agents, insulin sensitizers, lipid-lowering agents, and angiotensin II receptor antagonist [14,15]. Approximately 70% of patients with NASH have dyslipidemia

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[5,16]. Controlling dyslipidemia with diet, exercise, and lipid-lowering agents may help stabilize or reverse NAFLD. Atorvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl–coenzyme A reductase, has been reported to be effective in patients with NAFLD with dyslipidemia [17-20]. These reports have demonstrated that therapy with atorvastatin in NAFLD patients with dyslipidemia was effective for the reduction of serum aminotransferases and lipid levels. Kiyici et al [17] have demonstrated that the use of atorvastatin in NASH patients with hyperlipidemia was effective on the improvement of serum transaminases and liver density analyzed by computed tomography (CT). They have also stated that serum aminotransferases were normalized in approximately 60% of patients by the treatment of atorvastatin for 6 months, whereas those were normalized in approximately 20% of patients treated by ursodeoxycholic acid [17]. However, the efficacy of atorvastatin treatment for the histologic changes was not available. These observations let us to prove the effectiveness of atorvastatin in the treatment of NASH.

Therefore, in the present study, to evaluate the efficacy of long-term treatment of atorvastatin for NASH patients with hypercholesterolemia, we administered atorvastatin for 24 months to those who had failed to respond adequately to diet and exercise therapy; and we compared the resulting changes in clinical parameters, as well as the histologic changes.

2. Materials and methods

2.1. Patients

The prospective study included 31 patients with biopsy-proven NASH with dyslipidemia. Informed consent was obtained from each enrolled patient, and the study was conducted in conformity to the ethical guidelines of the 1975 Declaration of Helsinki [21] and was approved by the ethics and research committees of our hospital. In all patients, current and past daily alcohol intake was less than 20 g/wk; details regarding alcohol consumption were obtained independently by at least 2 physicians and confirmed by close family members. None of the patients had received any medication that could cause NASH [22]. In all of these patients, positive tests for the following disorders were excluded: secondary causes of steatohepatitis and drug-induced liver disease (eg, amiodarone [23] and tamoxifen [24]), alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, α_1-antitrypsin deficiency, hemochromatosis, Wilson disease, and biliary obstruction [22,25].

All patients received atorvastatin (10 mg/d) for 24 months. In addition, all patients were given standard weight-loss counseling and encouraged to follow a low-fat and low-carbohydrate diet before and during the treatment.

2.2. Clinical and laboratory evaluation

A complete physical examination was performed on each patient before and after treatment. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. *Obesity* was defined as a BMI greater than 25 kg/m^2, according to the criteria of the Japan Society for the Study of Obesity [26]. A CT scan was used to determine areas of visceral fat at the level of the umbilicus [27]. Hyperlipidemia was diagnosed for patients with cholesterol levels greater than 220 mg/dL and/or triglyceride level greater than 150 mg/dL. Hypertension was diagnosed if the patient was on antihypertensive medication and/or had a resting recumbent blood pressure of at least 130/85 mm Hg on at least 2 occasions.

Venous blood samples were taken in the morning after a 12-hour overnight fast. The laboratory evaluation in all patients included a blood cell count; and the levels of aspartate aminotransferase, alanine aminotransferase (ALT), γ–glutamyl transpeptidase, total cholesterol, triglyceride, fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), free fatty acid (FFA), hyaluronic acids, ferritin, and high-sensitivity C-reactive protein (CRP) were measured using the standard techniques of clinical chemistry laboratories before and after treatment. Adiponectin, leptin, tumor necrosis factor (TNF)–α, insulin, malondialdehyde (MDA), type IV collagen, and procollagen type III propeptide levels were measured before and after treatment, as previously reported [22].

A standard 75-g oral glucose tolerance test (OGTT) was performed on all patients before and after treatment. After a 12-hour fast, patients were given 75 g oral glucose solution. Plasma glucose and immunoreactive insulin were measured at 0, 30, 60, 120, and 180 minutes after the oral glucose load. Impaired glucose tolerance (IGT) was ascertained when at least 1 value was either greater than 110 mg/dL at 0 minute or greater than 140 mg/dL at 120 minutes, and diabetes was diagnosed at a 120-minute value of greater than 200 mg/dL according to the recently published recommendations of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [28]. Insulin resistance was calculated by the homeostasis model (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (in microunits per milliliter) × plasma glucose (in milligrams per deciliter)/405 [29].

Long-chain fatty acids in total plasma lipids were determined by using gas chromatography. In brief, total lipids were extracted from 0.5 mL of plasma using the method by Folch et al [30]; and isolated lipid fractions were prepared by transesterification under N_2 with 14% boron trifluoride in methanol at 100°C for 20 minutes. Fatty acid methyl esters were analyzed by gas chromatography using a Shimadzu gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan) with flame ionization detector, a 0.25-mm inner diameter, and a 30-m capillary column containing Omega-wax stationary phase (Supelco, Bellefonte, PA) as reported previously [31,32]. Peaks of fatty acid methyl esters were identified by comparing fatty acid retention times with standard mixtures of fatty acid methyl esters (Supelco). Fatty acids were quantified using heneicosanoic acid methyl ester (21:0) as internal standard. Sample measurements were carried out in triplicates. Twenty-four fatty acid species were

determined in this study (C12:0, C14:0, C14:1ω5, C16:0, C16:1ω7, C18:0, C18:1ω9, C18:2ω6, C18:3ω6, C18:3ω3, C20:0, C20:1ω9, C20:2ω6, C20:3ω9, C20:3ω6, C20:4ω6, C20:5ω3, C22:0, C22:1ω9, C22:4ω6, C22:5ω3, C24:0, C22:6ω3, and C24:1ω9). Based upon these results, total fatty acids (TFA), saturated fatty acids (SFA), total unsaturated fatty acids (TUFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were calculated.

2.3. Pathology

Patients enrolled in this study underwent a percutaneous liver biopsy under ultrasonic guidance using a 16-gauge SOLO CUT aspiration needle (Create Medic, Yokohama, Japan) when the informed consent was obtained. The mean length of the liver biopsy specimens was 30 ± 0.4 mm. Formalin-fixed, paraffin-embedded liver sections were stained routinely with hematoxylin-eosin, silver reticulin, Masson trichrome, Perls Prussian blue, and diastase-resistant periodic acid–Schiff. All the specimens were examined by an experienced pathologist who was unaware of the clinical and biochemical data of the patients. All cases of NASH were scored using the method of Brunt et al [33] as previously reported [22]. Steatosis was graded as follows: grade 1 (≥5% and <33% of hepatocytes affected), grade 2 (33%-66% of hepatocytes affected), or grade 3 (>66% of hepatocytes affected). Necroinflammation was graded 0 (absent) to 3 (1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild-to-moderate portal inflammation; 3, intraacinar inflammation and portal inflammation). Fibrosis was graded 0 (absent) to 4 (1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis). Ballooning was graded 0 (none) to 2 (1, few balloon cells; 2, many cells/prominent ballooning). The NAFLD activity score (NAS) was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2) as reported by Kleiner et al [34].

2.4. Statistical analyses

Results are presented as the medians and ranges for quantitative data or as numbers with percentages in parentheses for qualitative data. Statistical differences in quantitative data were determined using the Mann-Whitney *U* test and the Kruskal-Wallis test, when applicable. Fisher exact probability test was used for qualitative data. Correlation coefficients were calculated by Spearman rank correlation analysis. Differences were considered statistically significant at all *P* values less than .05.

3. Results

3.1. Patients enrolled

Thirty-one patients (20 male) of NASH with dyslipidemia, with a mean age of 52.5 ± 12.4 years, were enrolled in the study. The histologic findings before treatment are shown in Table 1. No patient had cirrhosis, and 26% had stage 3 fibrosis. Clinical and laboratory characteristics of enrolled patients are shown in Table 2. Body mass index ranged from 21.1 to 33.6 kg/m^2 and averaged 27.1 kg/m^2; 80.6% of enrolled patients had obesity according to the criteria of the Japan Society for the Study of Obesity [26]. Serum ALT levels ranged from 29 to 203 U/L and averaged 89.4 U/L. All patients had hypercholesterolemia, and 61.3% had concomitant hypertriglyceridemia. Six patients had fasting hyperglycemia, and 5 patients were diagnosed as diabetic by 75-g OGTT. A 75-g OGTT was performed in all enrolled patients. Twenty-nine percent of the patients showed normal glucose tolerance (NGT); 42%, IGT; and 29%, diabetes mellitus (DM).

Table 1
Histologic findings of patients with NASH

	NASH (N = 31)
Steatosis grade	
1	21 (68%)
2	8 (26%)
3	2 (6%)
Necroinflammatory grade	
1	22 (71%)
2	8 (26%)
3	1 (3%)
Fibrosis stage	
1	10 (32%)
2	13 (42%)
3	8 (26%)
4	0 (0%)
Ballooning score	
0	0 (0%)
1	23 (74%)
2	8 (26%)

Values are number (%).

3.2. Biochemical and metabolic responses

After 24 months of treatment, all patients showed a significant reduction of liver transaminase and γ–glutamyl transpeptidase levels (Table 3). Both AST and ALT levels were in the reference range in 23 patients (74.2%) after treatment. Serum ALT levels fell from an average of 89.4 U/L at baseline to 35.9 U/L at 24 months (individual changes of ALT levels are shown in Fig. 1). Mean BMI was 27.1 ± 2.7 kg/m^2 at baseline and 26.7 ± 2.9 kg/m^2 at 24 months ($P > .39$). Significant improvement of serum lipid profile is shown in Table 3. Serum total cholesterol levels decreased from 237 ± 39 mg/dL at baseline to 163 ± 32 mg/dL after treatment. Serum triglyceride levels decreased from 199 ± 90 to 132 ± 44 mg/dL. Serum high-density lipoprotein cholesterol levels increased from 50 ± 12 to 55 ± 12 mg/dL. Serum low-density lipoprotein cholesterol levels decreased from 147 ± 31 to 81 ± 27 mg/dL. Serum fasting glucose and HbA_{1c} levels did not change significantly before and after treatment.

Table 2
Clinical and laboratory characteristics of patients with NASH before treatment

Characteristic	NASH (N = 31)
Sex (male/female)	20/11
Age (y)	52.5 (27-68)
BMI (kg/m^2)	27.1 (21.1-33.6)
<25	6 (19.4%)
25-29	20 (64.5%)
≥30	5 (16.1%)
Obesity (%)	25 (80.6%)
Hypercholesterolemia	31 (100%)
Hypertriglyceridemia	19 (61.3%)
Hypertension	15 (48.4%)
Fasting glucose (>110 mg/dL)	6 (19.4%)
75-g OGTT	
NGT	9 (29.0%)
IGT	13 (42.0%)
DM	9 (29.0%)

Results are presented as numbers with percentages in parentheses for qualitative data and as medians and ranges for quantitative data.

Adipocytokines and serologic parameters before and after treatment are shown in Table 4. Plasma adiponectin levels were significantly increased by 25% and plasma TNF-α levels were significantly decreased by 43% at the end of the treatment. The leptin levels were not changed significantly both in male and female subjects. Free fatty acid levels did not change, and MDA levels were significantly decreased by 20%. The liver fibrosis markers type IV collagen and hyaluronic acid levels were decreased significantly. Ferritin levels decreased significantly. High-sensitivity CRP, a marker for inflammation, decreased significantly. Plasma glucose and insulin levels during glucose tolerance test showed high levels of postprandial glucose, insulin hypersecretion, and delayed peak of insulin secretion. These trends were not significantly changed by the treatment (at baseline and after treatment, 29% and 32%, 42% and 39%, and 29% and 29% of the patients showed NGT, IGT, and DM, respectively). Insulin resistance as determined by HOMA-IR tended to decrease, but statistical significance was not obtained.

Table 3
Clinical and laboratory characteristics of the patients with NASH before and after treatment

	Before treatment (N = 31)	After treatment (N = 31)
BMI (kg/m^2)	27.1 ± 2.7	26.7 ± 2.9
AST (U/L)	51.1 ± 23.3	25.8 ± 7.3**
ALT (U/L)	89.4 ± 46.3	35.9 ± 13.5**
γ–Glutamyl transferase (U/L)	87 ± 74	51 ± 16**
Bilirubin, total (mg/dL)	1.0 ± 0.6	1.0 ± 0.5
Bilirubin, direct (mg/dL)	0.2 ± 0.1	0.2 ± 0.1
Albumin (g/L)	4.6 ± 0.3	4.6 ± 0.3
Total cholesterol (mg/dL)	237 ± 39	163 ± 32**
Triglyceride (mg/dL)	199 ± 90	132 ± 44**
HDL cholesterol (mg/dL)	50 ± 12	55 ± 12*
LDL cholesterol (mg/dL)	147 ± 31	81 ± 27**
Fasting Glucose (mg/dL)	107 ± 17	107 ± 16
HbA_{1c}	5.6 ± 0.7	5.7 ± 0.7

Results are expressed as means ± SD. *P* values for qualitative data were calculated using Fisher exact probability test, and *P* values for quantitative data were calculated using Mann-Whitney *U* test. AST indicates aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* $P < .05$, compared with the values before treatment.

** $P < .001$, compared with the values before treatment.

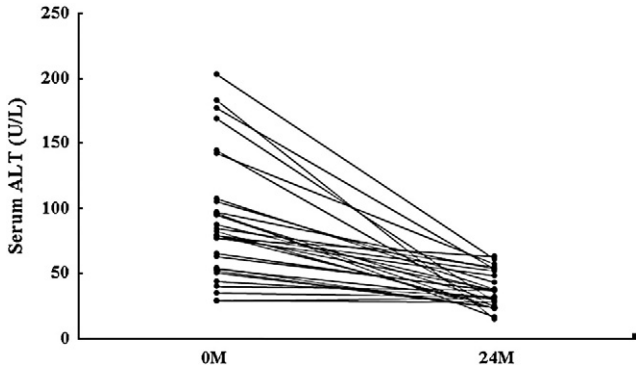

Fig. 1. Changes of serum ALT levels at baseline and after treatment with atorvastatin. (N = 31).

Table 4
Adipocytokines, serologic parameters, and radiological analyses of patients with NASH

	Before treatment (N = 31)	After treatment (N = 31)
Adiponectin (μg/mL)	5.3 ± 2.1	6.6 ± 2.4**
Leptin (ng/mL)	12.1 ± 10.8	9.8 ± 6.0
Male	7.3 ± 3.8	7.2 ± 2.6
Female	20.7 ± 13.9	15.0 ± 7.4
TNF-α (pg/mL)	17.2 ± 4.9	9.8 ± 5.3**
FFA (mEq/L)	0.5 ± 0.2	0.5 ± 0.2
MDA (nmol/mL)	0.5 ± 0.3	0.4 ± 0.2*
Type IV collagen (ng/mL)	4.4 ± 1.1	3.9 ± 0.8*
P-III-P (U/mL)	0.7 ± 0.3	0.6 ± 0.2
Hyaluronic acid (ng/mL)	41 ± 39	30 ± 25*
Ferritin (ng/mL)	247 ± 197	149 ± 111*
High-sensitivity CRP	0.15 ± 0.1	0.06 ± 0.05*
Visceral fat area (cm^2)	154 ± 20	139 ± 77
CT liver-spleen ratio	0.54 ± 0.25	0.97 ± 0.26**
HOMA-IR	3.46 ± 2.23	3.01 ± 1.62

75-g OGTT	Glucose (mg/dL)	IRI (mU/mL)	Glucose (mg/dL)	IRI (mU/mL)
0 min	100 ± 15	14 ± 7	107 ± 19	13 ± 5
30 min	186 ± 41	84 ± 48	185 ± 49	98 ± 98
60 min	212 ± 66	116 ± 63	211 ± 79	116 ± 72
120 min	178 ± 61	128 ± 80	183 ± 62	104 ± 43
180 min	115 ± 52	61 ± 44	135 ± 75	53 ± 41
ΣBSorΣIRI	757 ± 245	386 ± 189	820 ± 256	383 ± 151

Results are expressed as means ± SD. *P* values for qualitative data were calculated using Fisher exact probability test, and *P* values for quantitative data were calculated using Mann-Whitney *U* test. P-III-P indicates procollagen III N-terminal propeptide; IRI, immunoreactive insulin.

* $P < .05$, compared with the values before treatment.

** $P < .001$, compared with the values before treatment.

Long-chain fatty acids in total plasma lipids are shown in Table 5A. The concentrations of TFA, SFA, TUFA, MUFA, PUFA, n3-PUFA, and n6-PUFA were all elevated and decreased significantly after treatment. However, the ratios of SFA, TUFA, MUFA, PUFA, n3-PUFA, and n6-PUFA were not changed before and after treatment. The n-6/n-3 PUFA ratios were not significantly changed before and after treatment. When the fatty acid composition of serum total lipids was determined, a significant decrease was observed in C18:2,n-6 (linoleic acid, −21%) and C20:4,n-6 (arachidonic acid, −22%) (Table 5B).

Table 5
Fatty acid composition and concentration of plasma total lipids in patients with NASH before and after treatment

A. Fatty acid composition of plasma total lipids

	Before treatment, μg/mL (%)	After treatment, μg/mL (%)
TFA	3568 ± 635	2731 ± 293*
SFA	1266 ± 247	985 ± 155*
	(35.4% ± 1.7%)	(36.0% ± 2.7%)
TUFA	2303 ± 552	1746 ± 312*
	(25.8% ± 3.5%)	(25.8% ± 2.7%)
MUFA	924 ± 232	709 ± 138*
	(25.8% ± 3.5%)	(25.8% ± 2.7%)
PUFA	1378 ± 321	1037 ± 174*
	(38.8% ± 6.6%)	(38.2% ± 7.8%)
n-3 PUFA	313 ± 86	214 ± 96*
	(8.9% ± 2.7%)	(7.7% ± 2.6%)
n-6 PUFA	1064 ± 234	821 ± 78*
	(29.8% ± 3.9%)	(30.4% ± 5.2%)
n-6/n-3 PUFA ratio	3.39 ± 2.73	3.83 ± 0.82
Saturated-unsaturated FA ratio	0.55 ± 0.02	0.56 ± 0.03

B. Fatty acid concentration of plasma lipids

	Before treatment (μg/mL)	After treatment (μg/mL)
C12:0	1.7 ± 0.6	1.8 ± 0.4
C14:0	35.6 ± 12.1	31.5 ± 7.3
C14:1	0.1 ± 0.0	0.1 ± 0.0
C16:0	917.7 ± 177.9	698.1 ± 117.1
C16:1	92.8 ± 13.9	66.6 ± 12.8
C18:0	266.6 ± 45.0	217.6 ± 26.1
C18:1	784.0 ± 208.9	604.3 ± 120.6
C18:2	871.8 ± 189.3	666.0 ± 50.1*
C18:3	9.8 ± 3.5	11.4 ± 6.2
C18:3	30.4 ± 7.8	23.5 ± 8.5
C20:0	9.0 ± 1.8	7.8 ± 1.1
C20:1	6.7 ± 1.7	5.8 ± 1.8
C20:2	6.1 ± 1.3	5.1 ± 1.3
C20:3	1.4 ± 0.4	1.5 ± 2.6
C20:3	34.7 ± 8.3	30.0 ± 2.6
C20:4	137.8 ± 30.7	105.3 ± 17.1*
C20:5	100.7 ± 54.0	72.4 ± 54.9
C22:0	19.3 ± 4.5	15.2 ± 2.0
C22:1	3.1 ± 0.6	3.1 ± 0.5
C22:4	3.9 ± 1.4	3.1 ± 0.7
C22:5	24.7 ± 4.8	20.5 ± 10.9
C24:0	15.9 ± 5.1	12.8 ± 1.3
C22:6	157.1 ± 19.2	97.8 ± 21.5
C24:1	37.6 ± 6.7	29.4 ± 2.6

* $P < .05$, compared with the values before treatment.

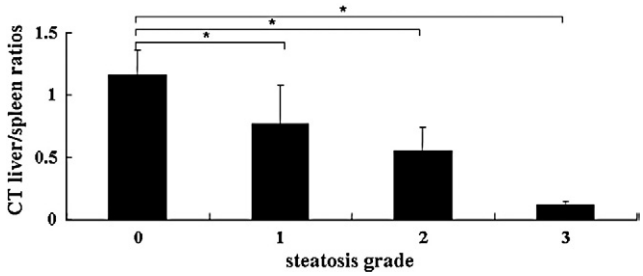

Fig. 2. Relationship between steatosis grade and liver density as measured by CT liver-spleen ratios. Results are expressed as means ± SD. *$P < .05$.

3.3. Liver density and plasma adipocytokines

Liver density was assessed by liver to spleen ratios as measured by means of abdominal CT scanning. Liver to spleen ratios were significantly increased from 0.54 ± 0.25 at baseline to 0.97 ± 0.26 at the end of treatment. Visceral fat area decreased from an average of 154 to 139 cm^2; however, statistical significance was not obtained. Liver density levels were inversely correlated with liver steatosis score (Fig. 2).

Table 6
Histologic changes before and after treatment

A. Histologic findings of patients with NASH

	Before treatment (n = 17)	After treatment (n = 17)
Steatosis grade	1.6 ± 0.1	0.8 ± 0.1**
Necroinflammatory grade	1.2 ± 0.1	1.0 ± 0.1
Fibrosis stage	1.8 ± 0.2	1.9 ± 0.2
Ballooning score	1.2 ± 0.1	1.0 ± 0.1
NAS	4.1 ± 0.3	2.9 ± 0.2**

B. Changes of distribution in grades and stages in patients with NASH

	Before treatment (n = 17)	After treatment (n = 17)
Steatosis grade		
0	–	4 (24%)
1	8 (47%)	12 (70%)
2	8 (47%)	1 (6%)
3	1 (6%)	–
Necroinflammatory grade		
1	13 (76%)	16 (94%)
2	4 (24%)	1 (6%)
3	–	–
Fibrosis stage		
1	6 (35%)	5 (29%)
2	8 (47%)	8 (47%)
3	3 (18%)	4 (24%)
4	–	–
Ballooning score		
0	1 (6%)	1 (6%)
1	11 (65%)	15 (88%)
2	5 (29%)	1 (6%)

Values are expressed as means ± SD (in micrograms per milliliter, n = 5). A. Values are expressed as means ± SD. B. Values are number (%).
** $P < .001$, compared with the values before treatment.

Changes of adiponectin levels were inversely correlated with those of steatosis grade ($P < .001$) and NAS ($P < .001$). Changes of TNF-α levels were positively correlated with those of steatosis grade ($P < .001$) and NAS ($P < .0001$). However, changes of leptin levels were not correlated with both steatosis grade and NAS.

3.4. Histologic responses

Follow-up liver biopsies were available on 17 patients. Table 6 shows the histologic changes before and after treatment. After treatment, macrovesicular steatosis, Mallory bodies, lipogranulomas, and NAS were improved significantly. Perisinusoidal, portal, and bridging fibroses were not changed. In brief, 13 patients (76%) had improvement and 4 had no change in NAS. Bridging fibrosis was found in 3 patients at baseline, and it vanished in 2 patients after treatment. Fibrosis stage increased in 4 patients (24%; 1 patient: from stage 1 to 2; 1 patient: from stage 1 to 3; 2 patients: from stage 2 to 3; before and after treatment) and did not change in 11 patients (65%).

4. Discussion

In this study, we assessed whether long-term treatment of atorvastatin would improve biochemical and histologic features of disease activity in NASH patients with dyslipidemia. All patients who received treatment with atorvastatin for 24 months showed an improvement or normalization of their serum lipid profiles. All 31 patients enrolled had improvements in serum aminotransferase levels. Alanine aminotransferase levels became normal in 74.2%. Imbalance of adipocytokines (reduced plasma adiponectin and increased plasma TNF-α levels), lipid peroxidation products (MDA levels), fibrosis markers (type IV collagen and hyaluronic acid levels), ferritin levels, and high-sensitivity CRP levels were significantly improved. Liver density was significantly improved or normalized without significant changes of visceral fat area. Plasma glucose levels, insulin levels, and total secretion amounts of glucose and insulin during OGTT were not affected by the atorvastatin treatment. Long-chain fatty acids in total plasma lipids were reduced significantly; reduction was specially evident in the n-6 series (C18:2,n-6 and C20:4,n-6). The histologic features of steatohepatitis (indicated by reduced score of NAS) were reduced. Whereas overall changes of fibrosis stage were not significantly changed, those were improved or not deteriorated in 76% of patients. Moreover, we found no significant elevation of liver enzymes during atorvastatin treatment; and no adverse effects were observed. Taken together, these results serve that atorvastatin has efficacy in patients with NASH accompanied by dyslipidemia.

Kiyici et al [17] have demonstrated the usefulness of atorvastatin in NASH patients with hyperlipidemia, and other reports have also demonstrated the improvement of liver enzymes in NAFLD patients with hyperlipidemia by atorvastatin [18-20]. Our study was in accordance with these reports and included multiple end point measurement (liver enzymes, adipocytokines, IR, lipid profile, glucose metabolism, and histologic changes before and after treatment). In experimental models, the decrease in hepatic triglyceride secretion without an increase in hepatic triglyceride concentration, the reduction of hepatic FFA, and the reduction of cholesteryl ester availability derived from newly synthesized cholesterol that limits the secretion of very low-density lipoprotein by statins including atorvastatin have been demonstrated [35,36]. Furthermore, a recent report by Kainuma et al [37] has shown that an animal fed a high-cholesterol diet exhibits hepatic steatosis, inflammation, ballooning, and fibrosis, histologic features of NASH. Thus, atorvastatin could be beneficial; and controlling the excess cholesterol might be useful for the treatment of NASH with dyslipidemia.

Oxysterols and other cholesterol oxidation products are physiologic ligands of nuclear liver X receptor (LXR). The LXR plays an important role in cholesterol homeostasis (serves as molecular sensors of cellular cholesterol concentrations and effectors of tissue cholesterol reduction), glucose metabolism, and fatty acid synthesis as well [38-40]. The LXR regulates lipogenic gene expression (eg, fatty acid synthase) by controlling sterol regulatory element–binding protein 1c (SREBP-1c) [38-40]. Several reports have demonstrated that activation of LXR leads to hepatic steatosis through activation of SREBP-1c in an animal model [41]. Furthermore, statins have been reported to decrease SREBP-1 [42,43]. Taken together, controlling cholesterol levels by statins, for example, atorvastatin, could be effective and reasonable in the treatment of NASH with dyslipidemia.

Another mechanism of atorvastatin is to induce peroxisome proliferator–activated receptor (PPAR) α and PPARγ [44-47]. The PPARα activation increases β-oxidation of fatty acids, followed by the decrease of fatty acids available for triglycerides synthesis, and thus decreases the content of triglycerides in the liver. The PPARγ has been demonstrated to attenuate the inflammatory response by inhibiting the production of TNF-α in monocytes [47]; to reduce interleukin-6 [46], a powerful inducer of CRP; and to reduce profibrogenic and proinflammatory actions in hepatic stellate cells [48,49]. These mechanisms are in accordance with our findings.

Significant reduction in high-sensitivity CRP (−40%) might be relevant because this marker of inflammation has the ability to activate, complement, and recruit monocytes and up-regulate adhesion molecules and chemoattractant chemokines [50]. Furthermore, atorvastatin has a potent antioxidant effect [51], thus influencing the pathogenesis of NASH and its metabolic abnormalities.

Another important finding in this study was the decrease of long-chain fatty acids in plasma lipids (Table 5). Especially, a significant decrease was observed in n-6 series linoleic acid and its metabolite arachidonic acid.

Prostaglandins derived from arachidonic acid have a modulatory role on interleukin-6 and TNF-α production, thus participating in the pathobiology of inflammation [52]. These changes of fatty acids may be one of the beneficial effects of this treatment.

Imbalance of adipocytokines (decreased levels of adiponectin and increased levels of TNF-α) was improved after treatment without significant changes of visceral fat. Atorvastatin did not change the glucose and insulin levels during 75-g OGTT before and after treatment. In other words, postprandial high glucose levels and hypersecretion of insulin were evident at the end of treatment. Because glucose and/or insulin directly influence connective tissue growth factor to induce fibrosis in hepatic stellate cells [53,54], this observation might explain the different results of liver fibrosis changes. In this regard, by addressing high levels of postprandial glucose and insulin, further improvement of histologic changes might be possible.

In conclusion, atorvastatin was administered to NASH patients with dyslipidemia who did not respond adequately to diet and exercise therapy. As a result, lipid levels, liver function, adipocytokines levels, fibrosis markers, long-chain fatty acid composition, and liver histologic findings were improved. However, 4 of 17 patients had progression of fibrosis over the 2-year period, with 3 of them progressing to stage 3. It is unclear whether this divergent response represents sampling error, heterogeneity in the population, or untreated postprandial hyperglyceridemia. Controlled trials are needed to further investigate and resolve this, and caution is warranted in applying statin therapy to NASH.

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