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The effect of atorvastatin on serum lipids and lipoproteins in patients with homozyous familial hypercholesterolemia undergoing LDL-apheresis therapy

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Abstract

The efficacy of atorvastatin, a new hydroxymethylglutaryl (HMG)-CoA reductase inhibitor, in reducing serum lipid levels, modifying lipoprotein composition, and suppressing cholesterol synthesis was evaluated in patients with homozygous familial hypercholesterolemia (homozygous FH) undergoing LDL-apheresis therapy. Atorvastatin was given in escalating doses (10, 20, and 40 mg/day) to nine patients with homozygous FH. Five of nine patients responded well to atorvastatin; four of these patients were receptor-defective and the remaining one was receptor-negative. The change in LDL-cholesterol in the receptor-defective patients averaged −20.6% compared to the baseline level at the highest dose of atorvastatin. Of five receptor-negative type patients, only one showed good response to atorvastatin therapy with a LDL-cholesterol reduction of 14.9%. Although the other four receptor-negative patients did not show a change in LDL-cholesterol, all of them exhibited a considerable increase in HDL-cholesterol. All patients showed reduced urinary excretion of mevalonic acid, suggesting that atorvastatin decreases LDL-cholesterol by inhibiting cholesterol biosynthesis even where LDL-receptor activity is not present. Atorvastatin also decreased serum triglycerides in both receptor-negative and defective patients, especially in the latter. As cholesterol level rebounds quickly after each apheresis procedure, a combination therapy using atorvastatin and apheresis may increase the efficacy of the apheresis treatment, improving cost-benefit effectiveness by reducing the frequency of the apheresis treatment.

Keywords: Familial hypercholersterolemia (therapy); Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor; Atorvastatin; Cholesterol metabolism; Serum cholestertol; LDL-aphersis

1. Introduction

Recently developed antilipidemic drugs such as HMG-CoA reductase inhibitors (statins) have enabled relatively easy treatment of most patients with heterozygous forms of familial hypercholesterolemia (heterozygous FH) [1,2]. However, individuals homozygous

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for FH show little response to any drug therapy. Although several special treatments have been tried, they are impractical due to low efficacy or expense. As examples, the portacaval shunt operation [3] showed little efficacy, while liver transplantation [4] remains impractical due to expense, undesirable host-graft immune responses and a shortage of donors. Although gene therapy may be available in the future [5], LDL-apheresis is the only currently available practical method to treat homozygous FH or other types of severe hypercholesterolemias [6,7]. LDL-apheresis diminishes anginal attacks shortly after the start of the therapy and atherosclerotic vascular lesions regress after 2–3 years [8–11]. Reports from several clinics show that the prognosis is good for several years if apheresis is repeated once every week or two [6,7,12,13]. However, long-term observation for more than 10 years revealed that complete prevention of coronary atherosclerosis took place in patients only if treatment started at an early stage of the disease [7,14]. Consequently, more aggressive therapeutic approach to lowering LDL-cholesterol is necessary.

Long-term observation in several laboratories has shown that aortic valve morbidity [15] is the most obstinate complication of FH, especially in homozygous FH patients. Although the development of atherosclerosis may depend on factors other than hypercholesterolemia itself, the marked rebound of serum cholesterol taking place after each apheresis procedure [16] seems to be a major determinant for the progression of debilitating complications such as aortic valvulopathy.

It is well known that statins do not effectively treat homozygous FH unless extremely large doses are used, because of the absence of functioning LDL-receptors in these patients [2,17,18]. Atorvastatin, a new HMG-CoA reductase inhibitor, is reportedly much more potent than previous statins [19,20]. It also has the added property of lowering plasma cholesterol by inhibiting secretion of apolipoprotein B (apo B)-containing lipoproteins from the liver [21,22]. Marais et al. gave atorvastatin to patients undergoing LDL-apheresis and found that atorvastatin enhanced the efficacy of LDL-apheresis and plasma exchange [23].

There are different cases of homozygous FH with different severities which depend upon the activity remaining in the mutant LDL-receptors. There is also a large individual variation in the rate of LDL synthesis and the flux of cholesterol into plasma, independent of differences in LDL-receptor activity [16,24]. In addition, serum LDL-cholesterol levels are strongly influenced by diet, for example, the average cholesterol levels of heterozygous FH patients in Japan are much lower than levels in patients of any ethnic groups living in Western countries [1,2]. Based on these informations, the effect of relatively high doses of atorvastatin on the efficacy of LDL-apheresis and the safety of statin therapy in homozygous FH patients were assessed in this study.

2. Subjects and methods

2.1. Patients

Nine homozygous FH patients undergoing LDL-apheresis treatments once every week or two were enrolled in this study. Five patients had a receptor-negative phenotype and four had a receptor-defective phenotype. The backgrounds of individual patients, including the LDL affinity binding to mononuclear cells and the site of mutation of the LDL-receptor genes, are shown in Table 1. The mean weight of the patients was 64.4 kg (range 49.8 ~ 79.0) and their body mass index (BMI) 25.5 (range 18.9 ~ 35.8). One patient (Patient 7) was extremely obese and the mean BMI of the other eight patients was 24.2 ($\pm$ 3.1). All the patients had been given a dietary advice by expert lipidologists or cardiologists already before they visited the apheresis clinics of our group. They or their mothers were recommended to take or to prepare a low-fat, low-calorie diet, with total fat less than 20% of the total energy, P/S ratio 1.3–1.5 and cholesterol less than 200 mg/day, even though the average fat intake of Japanese is still not so high (about 25% with P/S ratio 1.1–1.3) as in Western countries. Education to reduce the total calorie intake did not work well in Patient 7.

Among the nine patients, Patient 2 had relatively low serum cholesterol compared with homozygous FH patients in general. Although the site of mutation had not been identified, we gave a diagnosis of homozygous FH, because both of his parents had cholesterol levels corresponding to FH heterozygotes and the patient himself had cutaneous xanthomas from soon after birth. Such low total cholesterol level in homozygous FH patients was not unusual in Japan.

2.2. Preparation of atorvastatin used in this study

Crystalline form of atorvastatin was used as a drug for this trial. This preparation is 1.5 times greater in efficacy compared to the amorphous form; 40 mg/day dose of crystalline form is almost equivalent to 60 mg/day dose of amorphous form preparation. The highest dose selected in this study was 40 mg/day because the tolerability and safety of the drug had been established in Japanese heterozygous FH patients [25].

2.3. Therapy schedule

For the first 4 weeks of prechallenge observation, each patient remained on their conventional apheresis

Table 1
Patient demographies[a]

No.	Sex (age)	LDL receptor	Height (cm)/weight (kg)	Frequency of LDL-apheresis	TC[b] at first visit (mg/dl)	Regular antilipidemic agents	LDL-receptor activity [c]	LDL-receptor gene mutation
1	M (19)	Defective	172/79	LDL absorption method (every week)	852	Simvastatin cholestylamine probucol*	21%	Mutations in exon 7 (missense mutation) and/or in exon 8 (analysis was done on his sister)
2	M (16)	Defective	176/71	LDL absorption method (every 2 weeks)	504	Simvastatin probucol* ethyl eicosapentanoate cholestylamine	23%	Unknown
3	F (57)	Negative	151/54	LDL absorption method (every 2 weeks)	748	Pravastatin bezafibrate probucol*	0%	Unknown
4	F (17)	Negative	163/50	Double membrane filtration method (every 2 weeks)	672	Simvastatin probucol*	0%	Intron 12 splice-donor Site GT→GC
5	F (60)	Defective	148/55	LDL absorption method (every 2 weeks)	661	Pravastatin probucol ethyl eicosapentanoate	9%	Unknown
6	M (23)	Negative	161/75	Double membrane filtration method (every 10 days)	822	Simvastatin probucol*	0%	2-base deletion in exon 15
7	F (20)	Negative	143/73	Double membrane filtration method (every 10 days)	806	Simvastatin probucol	0%	Same as patient 6
8	M (17)	Defective	160/70	LDL absorption method (every 2 weeks)	589	Pravastatin probucol	25%	Compound hetero (two different mutation sites) in exon 18
9	M (18)	Negative	158/54	LDL absorption method (every 2 weeks)	700	Simvastatin cholestylamine probucol*	0%	Exon 11-intron 11splice junction GT→CT

[a] M: male; F: female; *: co-administrated during the trial.
[b] Serum total cholesterol.
[c] Activity on lymphocytes : % of the average value of normal individual.

regimen including any drug(s) prescribed. The next 4 weeks was a wash-out period, when the administration of all drug(s) except probucol was stopped to observe baseline plasma lipid levels due to LDL-apheresis alone or LDL-apheresis plus probucol. Probucol at a consistent dosage was permitted for use throughout the study period if the attending physician considered the use of this drug was necessary. After the washout period, patients were given atorvastatin, beginning with a 10 mg/day dose (Step 1; for 6–8 weeks). Doses were increased to the next step (Step 2, 20 mg/day, for 6–8 weeks and Step 3, 40 mg/day, for 8 weeks) if patients tolerated atorvastatin well. After tolerability was confirmed, the highest dose (40 mg/day) of the drug was maintained for an additional 12 weeks (Step 4) and the interval between LDL-apheresis treatments gradually lengthened during the last half of this step. The compliance of drug administration was checked by the physicians each time each patient visited the clinic. It was almost 100%, with an exception of a period in Patient 7 (75% in week 17 ~ 21) and Patient 3 (25% in week 32 ~ 34). The drug concentration in the blood was also monitored at the Laboratory of Yamanouchi Pharmaceuticals.

Patients were treated with LDL-apheresis using consistent conditions and intervals during the trial. When the intervals between treatments changed due to misalignment with a phase or step, the patients were kept at the same phase or step until the intervals were brought back to the original length of time and the effect of the irregular interval disappeared.

2.4. LDL-apheresis

LDL-apheresis was carried out using either the affinity chromatography technique with a dextran sulfate–cellulose column (Liposorber System MA-01;Kaneka Corporation; Osaka) or the double membrane filtration techniques (KM 8500 eqipped with EVAL-4A new type, Kuraray Co., Osaka, or Prasauto 1000 equipped with Plasmaflo, Asahi Medicals, Tokyo) [6,7]. A consistant volume of blood plasma (3000–6000 ml depending upon the individual patients) was treated at each treatment. Heparin was regularly used as an anticoagulant.

2.5. Lipoprotein and lipid analysis

Lipid values (total cholesterol:TC, HDL-cholesterol:HDL-C, and triglyceride:TG) were measured at the Special Research Laboratories (Tokyo), where the cholesterol assay was controlled using the CDC Lipid Standardization Program. TC and TG were measured enzymatically. HDL-C was measured in the supernatant after precipitation of apo B-containing lipoproteins by the heparin-Ca^{2+} method. Apolipoproteins (apo A-I, B, and E) were mesured by the turbidimetric immunoassay (TIA) system using reagents from Daiichi Pure Chemicals (Tokyo) [26,27] and Lp(a) using the Tint Elize Lp(a) kit (Biopool, Sweden) [28]. Lipoprotein fractionation was carried out by ultracentrifugation using a table-top apparatus (Beckmann, Germany) [29] and TC and TG in each fraction (VLDL, LDL, and HDL) were measured.

Serum lipid levels and apolipoproteins were measured just before the start of each apheresis treatment. These values were used to evaluate the effects of atorvastatin. Percent change from baseline values obtained at the end of the wash-out period was calculated from the average of the last two sample points in each phase or step. The effect of apheresis itself was regularly assessed by measuring plasma lipids before application of plasma to the affinity column or the second membrane filter at the start of treatment, and once again at the end of each apheresis treatment.

The cholesterol rebound curve was obtained twice for each patient; once at the end of the wash-out period (before the start of the atorvastatin administration) and again after 4 weeks of the highest dosing regimen (40 mg/day on atorvastatin). Blood samples were taken at 3, 6, 9, 18, 24, 32, 44, 56, 68 h after the apheresis treatment, once on Day 7, once between Day 8 and 14, and finally on Day 15. The data were analyzed applying a two-compartment model using non-linear regression program (Win NONLIN) [30].

LDL-cholesterol values (LDL-C) were calculated using the Friedewald equation [31]. Urinary excretion of mevalonic acid was measured at Teijin Bio Laboratories using acid extraction followed by gas chromatography-mass spectrometry [32]. LDL-receptor activity in lymphocytes was measured by flow-cytometry at Biomedical Laboratories (BML, Tokyo) [33].

2.6. Monitoring adverse events

Adverse events related to atorvastatin administration were monitored during the whole trial period. Clinical signs, symptoms and laboratory data (biochemical measurements of plasma components, hematology, and urinalysis) were checked at least every 4 weeks.

2.7. Statistical analysis

Paired t-test was used to evaluate the change of lipid levels before and after atorvastatin treatment.

3. Results

3.1. Changes in serum lipid levels

Changes in TC, LDL-C, HDL-C, and TG are summarized in Table 2. TC levels decreased as the dose of

atorvastatin escalated in receptor-defective patients ($P = 0.047$), but no change was seen in receptor-negative patients, except in one patient (Fig. 1). LDL-C levels changed in the same way as TC. There was a 15% decrease in one receptor-negative type patient (Patient 9). Except for this case, receptor-negative patients were non-responders. There was no relationship between either the weight or BMI and the extent of the LDL-C reduction.

HDL-C levels slightly increased in both receptor-defective and negative patients (Fig. 2). Although the change was not significant, considerable increases were observed in four out of five patients with receptor-negative type. TG levels decreased significantly ($P = 0.042$) at the end of Step 4 in receptor-defective patients, but the whole movement was not statistically significant (Fig. 3). Three out of five negative patients also showed a slight TG decrease during the course of the atorvastatin treatment, although the difference between the baseline level and the level at Step 4 (40 mg/day) was not significant in this group. These observed changes in serum lipid levels induced by atorvastatin were almost equal to or greater than changes induced by the regular treatment (LDL-apheresis combined with antilipidemic drug(s)) used before the start of this trial.

3.2. Changes in apolipoproteins (apo A-I and B)

Apo A-I level showed no changes in either receptor-negative (from 90.2 ± 6.8 mg/dl at the baseline to 91.8 ± 14.3 in Step 4) or receptor-defective patients (from 76.8 ± 8.7 to 74.3 ± 8.7). Apo B slightly decreased in receptor-defective patients (from 172.5 ± 142.8 to 142.8 ± 52.0 mg/dl), while a slight increase was observed in receptor-negative patients (from 242.6 ± 37.5 to 262.8 ± 77.4). These slight changes in apo A-I and B were not comparable to the change in lipids.

3.3. Changes in lipoprotein lipid concentrations

Changes in cholesterol levels in LDL fractions obtained by ultracentrifugation were comparable to the change in LDL-C calculated by Friedewald equation; 22.2% reduction was observed in receptor-defective patients, while the level increased by 11% on average in receptor-negative patients (Table 3). Cholesterol levels in the VLDL fraction showed a decrease and the levels in HDL fraction showed a considerable increase in receptor-negative patients. In contrast, no marked changes in HDL-C were observed in receptor-defective patients. Triglyceride levels showed decreases in both LDL and VLDL fractions in receptor-defective patients. Significant decreases ($P < 0.05$) of triglyceride in HDL and VLDL fractions were observed in receptor-negative patients, while it was counterbalanced by the increase in LDL-fraction in this group (Table 3). There was a significant correlation between percent changes in VLDL-C and VLDL-TG (Pearson's correlation coefficient = 0.794) in Step 4 compared to the baseline level.

3.4. Changes in Lp(a) levels

Lp(a) slightly increased from 13.6 ± 6.2 to 18.6 ± 11.3 mg/dl due to atorvastatin treatment. However,

Table 2
Serum lipid levels in homozygous familial hypercholesterolemia patients just before each apheresis treatment[a]

Serum lipids	LDL-receptor phenotype	*N*	Regular treatment mg/dl (%)[b]	Baseline phase (mg/dl)	Step 4 mg/dl (%)[b]	Paired t-test[c]
Total cholesterol	Negative	5	463 ± 101.4 (−0.4)	463 ± 74.7	470 ± 99.2 (1.3)	N.S.
	Defective	4	292 ± 80.4 (−4.5)	307 ± 82.8	255 ± 89.9 (−17.7)	$P<0.05$
LDL − cholesterol	Negative	5	393 ± 84.3 (−1.5)	396 ± 61.5	401 ± 83.9 (0.9)	N.S.
	Defective	4	249 ± 74.3 (−5.2)	262 ± 74.2	212 ± 86.7 (−20.6)	N.S.
HDL-cholesterol	Negative	5	39 ± 4.2 (1.1)	39 ± 3.1	42 ± 4.9 (7.1)	N.S.
	Defective	4	30 ± 7.9 (−4.5)	32 ± 5.1	34 ± 3.7 (7.5)	N.S.
Triglyceride	Negative	5	157 ± 92.1 (28.1)	138 ± 105.1	136 ± 94.4 (2.1)	N.S.
	Defective	4	66 ± 28.7 (12.0)	61 ± 23.7	46 ± 16.7 (−24.0)	$P<0.05$

[a] The effects of atorvastatin–apheresis combination therapy in receptor-negative and defective phenotype compared with the effects of regular treatment. Mean ± S.D.

[b] % Change from baseline.

[c] Pre- and post-treatment of atorvastatin.

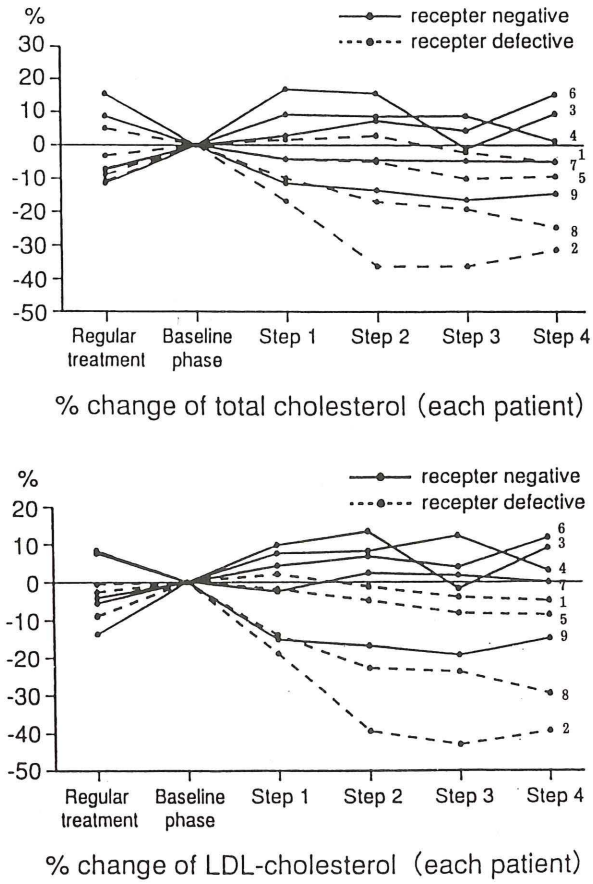

Fig. 1. Changes in total and LDL-cholesterol in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis by administration of atorvastatin. Atorvastatin was given in escalating doses from 10 to 40 mg/day (Step 1–4 as shown in the text: Subjects and methods (Section 2)). The patients were divided into receptor-negative (—●—) and -defective (···●···) groups and the percent changes of serum cholesterol from baseline levels are shown for individual patients.

since there was a wide range of individual variation, this difference was not statistically significant.

3.5. Urinary excretion of mevalonic acid

Decreases in urinary excretion of mevalonic acid were seen in 8 patients in whom the measurement was done, the average rate of change being −22.4% (Fig. 4). The most remarkable decrease was seen in Patient 9, whose LDL-C showed a good response in spite of the complete lack the receptor function. However, there was no significant correlation between urinary excretion of mevalonic acid and the percent change of either LDL-C nor VLDL-C in the whole group of patients.

3.6. LDL-receptor activity in lymphocytes

Affinity binding of LDL on LDL-receptors in lymphocytes was measured before and after the treatment of atorvastatin. However, there was no change in LDL-receptor activity even at the highest dose of the drug (data not shown).

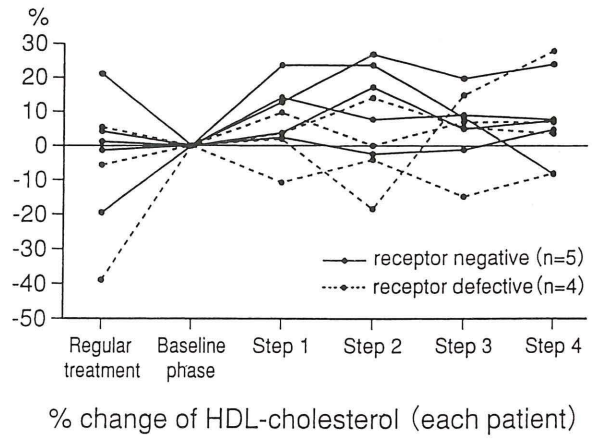

Fig. 2. Changes in HDL-cholesterol in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis by the administration of atorvastatin in escalating doses. The values for individual patients are shown: (—●—) receptor-negative, (···●···) receptor defective patients.

3.7. Analysis of the rebound curve of cholesterol after the apheresis treatment with and without atorvastatin

The rebound curve of cholesterol after the apheresis treatment was obtained in only one receptor-defective and four receptor-negative patients. It showed remarkable improvement after atorvastatin treatment in a receptor-defective patient (Patient 5), whose TC and LDL-C before the apheresis treatment decreased by 18 and 21%, respectively, by this assay.

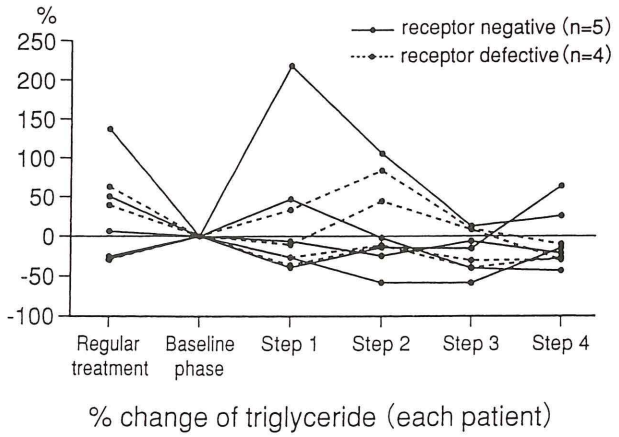

Fig. 3. Changes in serum triglyceride in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis by administration of atorvastatin in escalating doses. The values for individual patients are shown: (—●—) receptor- negative, (···●···) receptor-defective patients.

Table 3
Serum lipid (cholesterol and triglyceride) concentrations in lipoprotein fractions isolated by ultracentrifugation after atorvastatin (40mg/day, at the end of Step 4) treatment in receptor-negative and -defective homozygous familial hypercholesterolemia patients compared to the levels in baseline phase and regular treatment phase[a]

Lipoprotein fractions	Activity of LDL receptor	Number of subjects	Regular treatment phase	Baseline phase	End of Step 4[b]
LDL-cholesterol mg/dl (%)	Negative	5	391.0 ± 84.0 (3.7 ± 19.1)	376.3 ± 40.8	430.8 ± 101.4 (11.1 ± 23.6)
	Defective	4	249.8 ± 78.7 (−7.8 ± 10.8)	270.0 ± 74.0	216.0 ± 106.2 (−22.2 ± 22.4)
	Total	9	328.2 ± 106.7 (−1.4 ± 16.2)	329.1 ± 77.6	323.4 ± 149.7 (−5.5 ± 27.8)
VLDL-cholesterol mg/dl (%)	Negative	5	31.8 ± 30.5 (68.8 ± 137.7)	22.5 ± 19.5	13.8 ± 14.6 (−44.5 ± 34.2)(*)
	Defective	4	7.9 ± 5.2 (213.5 ± 402.5)	4.7 ± 4.7	4.5 ± 4.2 (19.6 ± 23.8)
	Total	9	21.2 ± 25.2 (133.1 ± 275.8)	14.6 ± 16.9	9.2 ± 11.1 (−12.4 ± 90.8)
HDL_2-cholesterol mg/dl (Δ mg/dl)	Negative	5	15.9 ± 2.7 (0.0 ± 3.8)	15.8 ± 4.5	21.6 ± 2.6 (4.5 ± 4.3)
	Defective	4	15.0 ± 2.4 (1.0 ± 5.1)	14.0 ± 4.9	17.1 ± 2.9 (3.1 ± 3.2)
	Total	9	15.5 ± 2.4 (0.5 ± 4.1)	15.0 ± 4.5	19.3 ± 3.5 (3.8 ± 3.6)
HDL_3-cholesterol mg/dl (Δ mg/dl)	Negative	5	16.2 ± 2.3 (2.0 ± 5.9)	14.2 ± 5.3	17.0 ± 3.0 2.9 ± 3.2)
	Defective	4	14.9 ± 3.8 (−1.5 ± 2.5)	16.3 ± 3.1	15.2 ± 1.2 (−1.1 ± 1.9)
	Total	9	15.6 ± 2.9 (0.5 ± 4.8)	15.1 ± 4.3	15.6 ± 3.0 (0.9 ± 3.2)
LDL-triglyceride mg/dl (%)	Negative	5	75.5 ± 43.0 (12.4 ± 45.5)	65.2 ± 24.7	81.9 ± 65.6 (13.5 ± 56.9)
	Defective	4	27.0 ± 10.3 (−6.2 ± 24.6)	32.9 ± 22.3	21.8 ± 10.4 (−25.6 ± 28.0)
	Total	9	53.9 ± 40.2 (4.1 ± 36.9)	50.8 ± 28.0	51.8 ± 54.1 (−6.0 ± 46.4)
VLDL-triglyceride mg/dl (%)	Negative	5	55.0 ± 44.7 (−27.3 ± 24.9)	86.6 ± 88.3	74.9 ± 95.2 (−34.0 ± 20.6) (*)
	Defective	4	16.5 ± 7.2 (−25.4 ± 47.2)	27.1 ± 14.8	13.9 ± 10.5 (−45.3 ± 44.5)
	Total	9	37.9 ± 37.8 (−28.4 ± 33.9)	60.1 ± 70.5	44.4 ± 70.7 (−39.7 ± 32.6) (**)
HDL-triglyceride mg/dl (%)	Negative	5	18.7 ± 14.0 (−42.9 ± 34.2)	81.5 ± 113.8	61.5 ± 68.5 (−28.5 ± 15.9) (*)
	Defective	4	9.0 ± 2.3 (15.3 ± 46.5)	8.6 ± 3.3	7.4 ± 3.3 (−14.4 ± 20.1)
	Total	9	14.4 ± 11.3 (−17.1 ± 48.3)	49.1 ± 89.2	34.4 ± 53.4 (−21.5 ± 18.4) (**)

[a] Mean ± S.D. (% change from baseline phase). *$P<0.05$ **$P<0.02$.
[b] In one receptor-negative patient, Step 3 data was used in place of the Step 4 data, because the sample at Step 4 was missed.

3.8. Adverse events

There were no serious adverse effects resulting in any patients stopping treatments. One patient complained numbness in her right hand, but drug administration continued without further problem. Some sporadic changes of clinical laboratory tests (elevation of ALT, AST, and/or CPK), possibly attributable to atorvastatin, were observed in four patients. However, these changes were mild (within double the upper limit of normal range) and disappeared during the course of the trial period. Atorvastatin was well tolerated in all patients.

4. Discussion

Homeostasis of cholesterol in both blood plasma and tissue cells is mediated primarily by LDL-receptors [34]. In FH patients, who lack this receptor function, plasma

LDL and cholesterol levels markedly increase. This situation is most severe in homozygous individuals [2,17]. Although the cholesterol level can be reduced by statins in heterozygous individuals [1,2], antilipidemic drugs, except probucol, show almost no effect against homozygous FH, because of the almost complete absence of receptor-mediated LDL removal from the plasma [2,17,35–37]. In the earliest report on the effect of compactin, the first HMG-CoA reductase inhibitor, an extremely large dose of the drug was slightly effective in reducing the serum cholesterol even in a homozygous FH patient of the receptor-negative phenotype [17]. However, the appearance of a serious adverse event involving muscular symptoms put strong limitations on the use of statins at a very high dosage. Recently, Raal et al. reported that a very high dose of simvastatin (80 or 160 mg/day) was successful in LDL reduction in homozygous FH including one receptor-negative type patient [18]. No serious adverse effects occurred during their study.

Atorvastatin is a second generation statin produced by chemical synthesis [38]. Reportedly, it has an additional cholesterol-lowering ability mediated by the suppression of VLDL secretion from the liver. This mechanism is independent of the LDL-receptor mechanism [21,22]. Most of the cholesterol-lowering effect of statins is mediated by the enhancement of LDL-receptor expression induced by even a small decrease in intracellular cholesterol concentration, especially in the liver [39]. Therefore, the additional lipid-lowering effect of atorvastatin seems to be a unique characteristic of this drug [40,41]. In addition, atorvastatin has a wider range of tolerability without the appearance of adverse effects than older statins [42]. Therefore, the drug can be used at a very high dose.

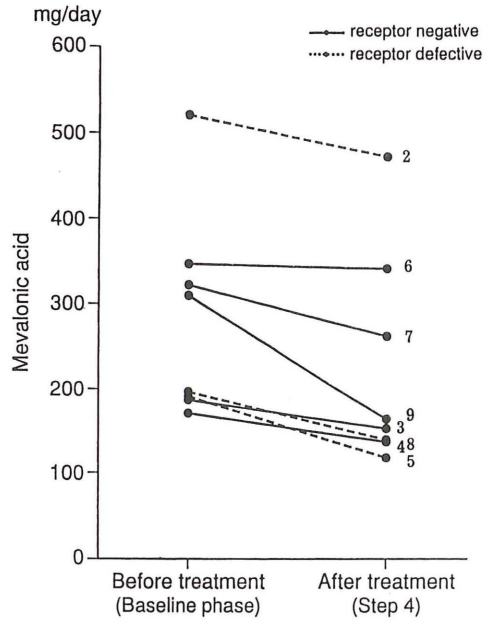

Fig. 4. Changes in a 24 h urinary excretion of mevalonic acid by the administration of atorvastatin at a dose of 40 mg/day. Values for individual patients are plotted.

Treatment of homozygous FH patients is now mainly done by using LDL-apheresis extracting LDL and other apo-B containing lipoproteins through an extracorporeal circulation system [6,7]. However, a marked rebound of cholesterol takes place after the apheresis treatment. Because of the almost complete lack of LDL-receptors and also the increase in synthesis of cholesterol, the rebound is very severe in homozygous FH patients [16], making it difficult to keep the LDL-cholesterol level in an optimal range where the development of atherosclerosis can be minimized [16,30]. Even by repeating the apheresis treatment once a week, the progression of atherosclerotic vascular lesions cannot be prevented completely.

A recent report from South Africa demonstrated that high doses of atorvastatin (amorphous type, 80 mg/day) was effective in suppressing cholesterol synthesis and keeping LDL-C levels lower in patients with homozygous FH compared with the conventional apheresis treatment without drugs [23]. However, most of the subjects of that study had a receptor-defective phenotype peculiar to South Africa [43]. Therefore our experiments tested the generality of this result regardless of the type of LDL-receptor mutation and also in ethnic populations with different dietary conditions.

Atorvastatin was effective in suppressing cholesterol rebound after apheresis mainly in receptor-defective individuals. In subjects with residual LDL-receptor activity, statin may act by increasing the expression of the receptor protein. Interestingly, there was a decrease in VLDL-C and -TG in patients with receptor-negative type FH. In addition, the increase in HDL-C was greater in receptor-negative than in receptor-defective patients. Atorvastatin also effectively reduced LDL-C in one receptor-negative patient. The excretion of mevalonic acid in urine was markedly decreased in this patient, while the decrease was seen in general irrespective of the molecular types of the receptor mutation. These results indicate that both cholesterol synthesis and secretion of VLDL were suppressed by atorvastatin even in patients who completely lacked LDL-receptors.

Because of the large content of cholesterol in both tissue and plasma, it may be difficult to reduce total plasma cholesterol significantly in a short period of time by suppressing cholesterol synthesis without the up-regulation of LDL-receptors. The presence of lecithin-cholesterol acyl transferase (LCAT) and cholesteryl ester transfer protein (CETP) increases esterified cholesterol content of LDLs [44,45]. Consequently, there should be high amounts of esterified cholesterol in LDL fraction in patients with a complete lack of LDL-receptors. Even if cholesterol is extracted from the tissue by HDL, cholesterol will stay as a

constituent of LDL due to the presence of LCAT and CETP and the absence of LDL-receptors. However, the suppression of cholesterol synthesis will certainly give advantages to homozygous FH patients such as improvement of clinical symptoms or, at least, slowing the progression of vascular lesions, as previously shown using large doses of compactin [17]. Since homozygous FH is very severe, the suppression of cholesterol synthesis even to a small extent is beneficial. Further clinical approach using expanded-dose can be tried in the future.

5. Conclusion

(1) Five of nine homozygous FH patients responded well to atorvastatin. Four patients had a receptor-defective and one was receptor-negative phenotype.

(2) LDL-C decreased with escalating doses of atorvastatin. The change at the highest dose was 20.6% in receptor-defective patients. In receptor-negative patients, marked changes were not seen except for one patient showing a good response (− 15%).

(3) VLDL-C and VLDL-TG were decreased and HDL-C increased in receptor-negative patients. The decrease in TG was observed in both LDL and VLDL fractions in receptor-defective patients. Changes in LDL-C, HDL-C, and TG due to atorvastatin were much greater than those observed in previous drug treatments combined with LDL-apheresis in four homozygous patients including one receptor-negative individual.

(4) All patients showed a reduced urinary excretion of mevalonic acid, while there was no change in LDL-receptor activity in lymphocytes. This suggests that atorvastatin is able to decrease TC by inhibiting the biosynthesis of cholesterol in the liver even in the absence of increased LDL-receptor gene expression.

(5) Percent changes of LDL-C and apo B and that of VLDL-TG and VLDL-C correlated significantly. These findings (items 3–5) suggest that the decrease in LDL-C is mainly attributable to the presence of remnant LDL-receptor activity in receptor-defectiive patients, while the reduction of the VLDL secretion from the liver gave a beneficial effect in receptor-negative patients.

(6) Atorvastatin was well tolerated and effective in homozygous FH patients undergoing LDL-apheresis.

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