Efficacy of colestimide coadministered with atorvastatin in Japanese patients with heterozygous familial hypercholesterolemia (FH)

Circ J 2005; **69:** 515–520

Efficacy of Colestimide Coadministered With Atorvastatin in Japanese Patients With Heterozygous Familial Hypercholesterolemia (FH)

Masa-aki Kawashiri, MD; Toshinori Higashikata, MD; Atsushi Nohara, MD; Junji Kobayashi, MD*; Akihiro Inazu, MD**; Junji Koizumi, MD†; Hiroshi Mabuchi, MD

Background Colestimide, a 2-methylimidazole-epichlorohydrin polymer, is a new bile-acid-sequestering resin, that is 4-fold as powerful at lowering low-density lipoprotein cholesterol (LDL-C) as the conventional resin (cholestyramine). Moreover, colestimide has excellent patient compliance because it is available in tablet form.

Methods and Results The clinical efficacy of colestimide coadministered with atorvastatin on lipid and apolipoprotein concentrations was examined in 15 patients (M/F=10/5, mean±SE age=54±9 years) with heterozygous familial hypercholesterolemia (FH). After a period of wash-out of any lipid-lowering drugs, atorvastatin (20–40 mg) was administered to patients for at least 8 weeks, and then 3 g of colestimide was administered for a further 8 weeks. Total and LDL-C significantly (<0.0001) decreased by 35% from 361 to 233 mg/dl and 41% from 274 to 161 mg/dl, respectively. Addition of colestimide caused a further significant 12% and 20% reduction, respectively, from the initial values to 205 and 129 mg/dl, respectively. Colestimide was also effective in reducing serum LDL-C concentrations in heterozygous FH patients with hypertriglyceridemia (triglycerides ≥150 mg/dl).

Conclusions When monotherapy with atorvastatin is insufficient to treat severely hypercholesterolemic patients, such as those with heterozygous FH, colestimide acts to reinforce the action of statins. (*Circ J* 2005; **69:** 515–520)

Key Words: Atorvastatin; Colestimide; Familial hypercholesterolemia

Familial hypercholesterolemia (FH), is an autosomal dominant disorder that is attributable to a mutated low-density-lipoprotein (LDL) receptor gene, and is characterized by excessively high concentrations of LDL cholesterol (LDL-C), tendon xanthomas, and premature coronary artery disease (CAD)[1] If cholesterol lowering therapy is ineffective, as it is in more than 70% of cases of heterozygous FH in Japan, the patient dies from atherosclerotic cardiovascular disease. The mean age at death is 54 years for men, and 69 years for women[2] FH is one of the most common disorders causing coronary artery disease at the age of 40 years or less in Japan[3] Many clinical trials have proven the efficacy of cholesterol-lowering therapy using 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors, statins, for the primary and secondary prevention of CAD[4–9] It is also recognized that the increased use of cholesterol-lowering drugs, especially statins, is associated with improved cardiovascular prognosis of FH[10] The administration of statins increases the activity of LDL receptors,[11] thus for heterozygous FH patients whose LDL receptor activity is partially impaired, statins are the most effective cholesterol-lowering drugs. Recently, statins such as pravastatin and simvastatin have been superceded in Japan by atorvastatin, which is even more effective in reducing LDL-C. However, because FH is highly refractory to cholesterol-lowering drugs, monotherapy using atorvastatin frequently fails to achieve the target concentrations of LDL-C recommended by the Japan Atherosclerosis Society,[12] the Joint Task Force of European and other Societies[13] and the National Cholesterol Education Program in the United States of America[14] for the primary and secondary prevention of atherosclerotic cardiovascular disorders.

Bile-acid-sequestering resins interrupt the enterohepatic circulation of bile acids, resulting in the upregulation of LDL receptors on hepatocytes, especially when used in conjunction with statins[15,16] Colestimide, a 2-methylimidazole-epichlorohydrin polymer, is a new bile-acid-sequestering resin produced by the Mitsubishi Chemical Corporation, Tokyo, Japan (Fig 1). Its in vitro bile-acid-binding capacity is 4-fold greater than that of the conventionally used bile-acid-sequestering resin, cholestyramine[17] In addition, the clinical use of cholestyramine is limited because of poor patient compliance, mainly because the drug has to be dissolved in water before being taken. In the present study, we examined for the first time the combined effects of

(Received September 30, 2004; revised manuscript received January 24, 2005; accepted January 31, 2005)

*Molecular Genetics of Cardiovascular Disorders, Division of Cardiovascular Disease, Graduate School of Kanazawa University, *Department for Life-style-related Diseases, Graduate School of Kanazawa University, **School of Health Science, Faculty of Medicine and †Department of General Medicine, Kanazawa University Hospital, Kanazawa University, Kanazawa, Japan*

Mailing address: Masa-aki Kawashiri, MD, Molecular Genetics of Cardiovascular Disorders, Division of Cardiovascular Disease, Graduate School of Medical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. E-mail: masaaki@im2.m.kanazawa-u.ac.jp

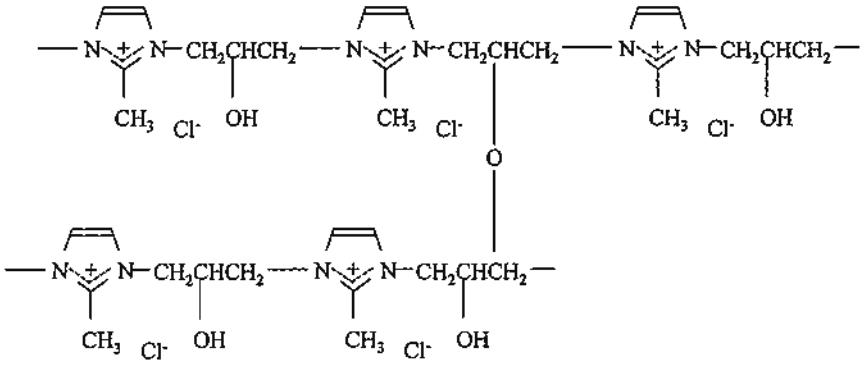

Fig 1. Producing a hypolipidemic effect using cholestyramine, a conventionally used bile-acid-sequestering resin, requires the administration of large doses (9–27 g) because of its insolubility in water and because of the inaccessibility of bile acid to binding sites on the drug. Colestimide is a new bile-acid-sequestering resin developed by the Mitsubishi Chemical Corporation (Tokyo, Japan). It has an imidazolium salt on an epoxide polymer skeleton and exhibits clinical pharmacological effects similar to those of cholestyramine at approximately 1-quarter of its dose. It thus has excellent patient compliance.

Table 1 Effects of Colestimide Puls Atorvastatin on Lipid and Apolipoprotein Concentrations in Heterozygous Familial Hypercholesterolemia

	Baseline	*Atorvastatin 4 week*	*Atorvastatin*	*Atorvastatin + colestimide*	*p value*			
					ANOVA	*Baseline vs atorvastatin*	*Baseline vs atorvastatin + colestimide*	*Atorvastatin vs atorvastatin + colestimide*
Total cholesterol (mg/dl)	*361±51*	*242±45*	*233±35*	*205±30*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0011*
LDL cholesterol (mg/dl)	*274±59*	*168±45*	*161±31*	*129±23*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
HDL cholesterol (mg/dl)	*50±13*	*54±13*	*53±15*	*54±14*	*0.7542*	*0.5736*	*0.3504*	*0.6521*
Triglycerides (mg/dl)	*152±99*	*103±52*	*116±65*	*98±56*	*0.1448*	*0.0454*	*0.0076*	*0.2085*
Apolipoprotein (mg/dl)								
A I	*127±23*	*134±26*	*140±26*	*141±23*	*0.2351*	*0.0290*	*0.0674*	*0.6468*
A II	*35±5*	*37±7*	*36±6*	*30±4*	*0.0050*	*0.4130*	*0.0069*	*0.0056*
B	*211±34*	*134±31*	*133±23*	*108±18*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
C II	*5.9±2.1*	*4.6±1.8*	*4.8±1.5*	*4.0±1.4*	*0.0231*	*0.0012*	*<0.0001*	*0.0173*
C III	*12.6±4.0*	*10.3±3.5*	*11.5±3.6*	*10.3±3.2*	*0.2114*	*0.0959*	*0.0206*	*0.4530*
E	*7.3±1.7*	*5.1±1.2*	*5.1±0.8*	*4.5±1.0*	*<0.0001*	*0.0001*	*<0.0001*	*0.0450*

Values are mean±SE.

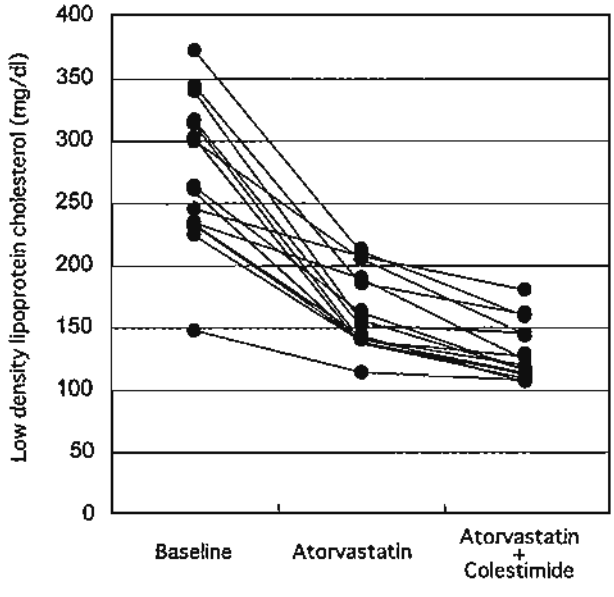

Fig 2. Effects of colestimide on low-density lipoprotein cholesterol (LDL-C) concentrations in patients with heterozygous familial hypercholesterolemia (FH) when administered in conjunction with atorvastatin. Fifteen patients with FH were administrated 20–40 mg of atorvastatin at least for 8 weeks, and then given an additional 3 g of colestimide for a further 8 weeks. This treatment regime resulted in a significant ($p<0.0001$) reduction in LDL-C from baseline levels, namely from 274 to 161 mg/dl (–41%; atorvastatin alone), and to 129 mg/dl (–53%; atorvastatin+colestimide).

colestimide and atorvastatin on lipid and apolipoprotein concentrations in Japanese patients with heterozygous FH.

Methods

Study Patients

The study population comprised of 15 patients (10 men, 5 women, mean±SE age 54±9 years) with heterozygous FH. All of the patients fulfilled our diagnostic criteria for FH:[2,18,19] primary hypercholesterolemia (>230 mg/dl) with tendon xanthomas, or first generation relatives of previously diagnosed heterozygous FH patients showing primary hypercholesterolemia (>230 mg/dl). All except for 1 participant had the mutation of the LDL receptor gene.[20] The mean±SE body mass index of the patient cohort was 24.5±2.5 kg/m^2. Stable CAD was already documented in 10 patients (67%), and none of them had had a recent acute coronary event. None had been diagnosed with cerebral atherosclerotic vascular disease. Five patients (33%) had impaired glucose tolerance; 1 was taking hypoglycemic agents, and had glycohemoglobin concentrations <7.0%, which varied by only ±1.0% during the study period. Written informed consent to participate in the study was obtained from each patient before entry into the study, and the ethical committee of Kanazawa University Hospital approved the study protocol.

Study Protocol

All the patients were outpatients at the beginning of the study. Any lipid-lowering agents were washed-out over a period of at least 4 weeks. Subjects taking probucol were excluded because its cholesterol-lowering effects continue

Table 2 Effects of Colestimide Plus Atorvastatin on Lipid and Apolipoprotein Concentrations in Heterozygous Familial Hypercholesterolemia (Subgroup Analysis)

	Baseline	Atorvastatin	Atorvastatin + colestimide	p value			
				ANOVA	Baseline vs atorvastatin	Baseline vs atorvastatin + colestimide	Atorvastatin vs atorvastatin + colestimide
Baseline triglyceride <150 mg/dl (n=10)							
Total cholesterol (mg/dl)	372±44	232±34	202±27	<0.0001	<0.0001	<0.0001	0.0032
LDL cholesterol (mg/dl)	300±47	165±30	129±22	<0.0001	<0.0001	<0.0001	0.0001
HDL cholesterol (mg/dl)	52±13	57±16	54±15	0.7422	0.2794	0.6393	0.5050
Triglycerides (mg/dl)	95±34	93±50	73±18	0.3410	0.9021	0.0258	0.2265
Apolipoprotein (mg/dl)							
A I	126±19	146±25	139±26	0.1695	0.0544	0.1170	0.7745
A II	35±5	36±7	29±4	0.0175	0.5091	0.2020	0.0080
B	212±33	126±20	103±12	<0.0001	<0.0001	<0.0001	0.0007
C II	4.8±1.4	4.3±1.5	3.4±1	0.0534	0.0353	0.0011	0.0326
C III	10.4±2.6	10.8±3.8	9.5±2.8	0.6800	0.8012	0.2784	0.3213
E	6.6±0.9	4.9±0.7	4.4±0.7	<0.0001	0.0002	<0.0001	0.0279
Baseline triglyceride ≥150 mg/dl (n=5)							
Total cholesterol (mg/dl)	338±60	233±41	212±37	0.0026	0.0035	0.0037	0.2050
LDL cholesterol (mg/dl)	222±44	162±37	130±28	0.0067	0.0118	0.0076	0.0420
HDL cholesterol (mg/dl)	47±15	44±10.0	54±12.4	0.4449	0.7258	0.4286	0.0576
Triglycerides (mg/dl)	268±81	161±74	148±75	0.0577	0.0004	0.0297	0.6651
Apolipoprotein (mg/dl)							
A I	131±31	127±27	144±17	0.5669	0.3521	0.3508	0.1334
A II	35±4	35±5	31±4	0.3687	0.6004	0.2367	0.2267
B	209±41	151±21	116±26	0.0022	0.0011	0.0016	0.0112
C II	8.0±1.4	6.1±0.8	5.4±1.4	0.0183	0.0058	0.0093	0.2366
C III	17.0±1.7	13.3±2.4	12.0±3.7	0.0362	0.0051	0.0300	0.7636
E	8.6±2.4	5.5±0.9	4.7±1.6	0.0155	0.0172	0.0046	0.3451

Values are mean±SE.

for more than 12 weeks. The subjects were initially administered atorvastatin as a daily dose of 10 or 20 mg in the evening; this was increased to 20–40 mg for at least 8 weeks, if serum concentrations of LDL-C were 30% over the target concentrations indicated by the Japan Atherosclerosis Society guideline![12] In Japan, the permitted maximum dose of atorvastatin is 40 mg/day; the mean dose administered in the present study was 32.0±8.6 mg. Colestimide (3 g) was added to atorvastatin monotherapy twice per day, before meals, once in the morning and once in the evening, for a further 8 weeks. Once colestimide treatment was started, the dose of atorvastatin was unaltered for any of the subjects throughout the remainder of the study period. Blood samples were obtained after an overnight fast at various time points during the study period.

Laboratory Procedures

Serum concentrations of cholesterol and triglycerides (TG) were determined by an enzymatic method, and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by a polyamine-polymer/detergent method (Daiichi, Tokyo, Japan)![21] LDL-C concentrations were determined by a direct method (Daiichi)![22] Serum concentrations of apolipoprotein AI, AII, B, CII, CIII, and E were determined as described previously![23]

Statistical Analyses

Values are expressed as the mean±SE unless stated otherwise. The effects of drug therapy on each variable were compared by means of single-factor analysis of variance (ANOVA), and then compared by the paired t-test when significant differences were observed. All statistical analyses were performed with the Stat View 4.5 system (Abacus Concepts, Berkeley, CA, USA). A p-value of less than 0.05 was considered to be statistically significant.

Results

Serum Total Cholesterol, LDL-C, and HDL-C (Table 1, Fig 2)

Administration of atorvastatin significantly (p<0.0001) decreased total cholesterol (TC) and LDL-C concentrations, from 361 to 233 mg/dl (–35%) and from 274 to 161 mg/dl (–41%), respectively. They decreased further (p=0.0011, and p<0.0001, respectively) from the baseline levels following the addition of colestimide, to 205 (–43%) and 129 (–53%), respectively. Atorvastatin monotherapy and atorvastatin plus colestimide increased HDL-C concentrations from 50 to 53 mg/dl (+6%) and 54 mg/dl (+8%), respectively. However, these changes were not statistically significant.

Triglycerides (Table 1)

Atorvastatin treatment effected a significant (p=0.0454) reduction in serum TG concentrations, from 152 to 116 mg/dl (–24%). Additional use of colestimide decreased the concentrations further (to 98 mg/dl, –36%), but this change was not statistically significant.

Apolipoprotein (Table 1)

Atorvastatin treatment resulted in increases in the mean concentrations of apolipoproteins AI (10%) and AII (4%). When colestimide was administered in conjunction with atorvastatin, the serum concentrations of apolipoprotein AI did not change; the mean concentrations of apolipoprotein AII, however, decreased by 15%. Monotherapy with atorvastatin caused significant reductions in the mean concentrations of apolipoproteins B (by 37%, p<0.0001) and E (by 30%, p<0.0001). Combined treatment with atorvastatin and colestimide caused further significant reductions in the concentrations of apolipoproteins B (by another 12%,

Table 3 Alterations in Liver Enzymes, Creatine Kinase, and Glycohemoglobin During Atorvastatin Monotherapy and in Combined Therapy With Colestimide

	Baseline	Atorvastatin	Atorvastatin + colestimide
AST (IU/L)	22±8	25±5	24±4
ALT (IU/L)	24±17	25±20	29±13
γGTP (IU/L)	69±73	74±66	52±47
CK (IU/L)	124±73	133±76	142±75
HemoglobinA1c (%)	5.3±0.5	5.5±0.6	5.3±0.6

Values are mean±SE.

p<0.0001) and E (by another 8%, p=0.045). The mean concentrations of apolipoproteins CII and CIII were also reduced with both atorvastatin monotherapy and atorvastatin in conjunction with colestimide.

Effects of Colestimide in Patients With Hypertriglyceridemia (Table 2)

Because bile-acid-sequestering resin is known to increase LDL receptor activity via a reduction in the cholesterol concentrations in hepatocytes, it may increase the production rate of very-low-density-lipoprotein, which contains a considerable amount of TG.[16] We therefore examined the effects of colestimide on the serum concentrations of lipids and apolipoproteins in 5 patients (M/F=2/3) with FH accompanied by hypertriglyceridemia (TG ≥150 mg/dl). Administration of atorvastatin alone significantly decreased TC and LDL-C concentrations from 338 to 233 mg/dl (–31%, p=0.0035) and from 222 to 162 mg/dl (–27%, p=0.0118), respectively. Although the doses of atorvastatin administered to these patients were greater than those administered to normotriglyceridemic FH patients (38.0±4.5 mg/dl vs 29.0±8.8 mg), the rates of reduction of both TC and LDL-C concentrations were less in the former than in the latter. They decreased further (p=0.2050, p=0.0420) from baseline following the addition of colestimide, to 212 mg/dl (–37%) and 130 mg/dl (–41%), respectively. Serum TG concentrations were significantly (p=0.0004) decreased by atorvastatin treatment, from 268 to 161 mg/dl (–40%). The addition of colestimide decreased serum TG by a further 5% (to 148 mg/dl).

Adverse Events

Atorvastatin was well tolerated, and all 15 patients were given the additional 3 g colestimide treatment. None of the patients suffered any severe adverse events that caused discontinuance of colestimide, and no abnormalities in the laboratory findings were observed, including significant elevations in hepatic enzyme (aspartate aminotransferase, alanine aminotransferase, and gamma glutamyl transpeptidase) or in creatine kinase (Table 3). Neither atorvastatin nor colestimide caused any impairment in glucose metabolism, as assessed using hemoglobinA1c (Table 3).

Discussion

In this study, we examined the effects of combined treatment with atorvastatin and colestimide on lipid concentrations in Japanese patients with heterozygous FH. Several studies have shown that when monotherapy with a statin is insufficient, combination therapy using a statin plus another lipid-lowering-drug can be effective.[15,24–27] This is the first report to examine the lipid-lowering effects of colestimide when used with a statin. The reductions in the lipid and apolipoprotein concentrations after 4 weeks of atorvastatin monotherapy were not significantly different to those after 8 weeks, which suggested that the LDL-C lowering effect had reached a plateau at 8 weeks. Thus, we can presume that the additional 20% of LDL-C lowering was caused by colestimide. Because FH is highly resistant to therapy with cholesterol-lowering drugs, with the frequent result of death from atherosclerotic cardiovascular disorders, the development of aggressive cholesterol-lowering therapies for FH patients is crucial. Although atorvastatin is one of the most effective statins in reducing serum LDL-C, monotherapy has frequently failed to achieve the target concentrations of LDL-C for the primary and secondary prevention of atherosclerotic cardiovascular disorders recommended by the Japan Atherosclerosis Society,[12] the Joint Task Force of European and other Societies[13] and the National Cholesterol Education Program in the United States of America.[14]

In the current study, LDL-C concentrations were reduced to less than 160 mg/dl by atorvastatin monotherapy in 9 (60%) and by atorvastatin plus colestimide in 14 (93%) patients (<160 mg/dl is the target concentration for the primary prevention of atherosclerotic cardiovascular disorders of patients without any coronary risk factors (Japan Atherosclerosis Society guideline category A)). On the other hand, in only 1 (7%) of the study subjects did atorvastatin monotherapy result in a reduction in serum LDL-C concentration to less than 120 mg/dl (Japan Atherosclerosis Society guideline category B3 and B4), which is the target concentration for the primary prevention of atherosclerotic cardiovascular disorders for patients with multiple coronary risk factors. However, this target concentration was reached in 8 (53%) of the subjects following the addition of colestimide. Thus, our results suggest that therapy with atorvastatin plus colestimide is suitable for the primary prevention of atherosclerotic cardiovascular disorders associated with heterozygous FH. However, serum LDL-C concentrations never reached less than 100 mg/dl in any of the study subjects, which is the target concentration for the secondary prevention of atherosclerotic cardiovascular disorders (Japan Atherosclerosis Society guideline category C), even with the combination therapy. More aggressive cholesterol-lowering therapy, such as LDL apheresis, is required to reach this target.[28]

In the current study, we examined the serum lipid and apolipoprotein concentrations of patients with heterozygous FH with hypertriglyceridemia (TG ≥150 mg/dl). FH patients with hypertriglyceridemia showed lower LDL-C and HDL-C concentrations, and tended to be treated with higher doses of atorvastatin, than did normotriglyceridemic FH patients. Atorvastatin monotherapy significantly (p=0.0001) decreased serum TG concentrations in FH patients with hypertriglyceridemia. Importantly, atorvastatin plus colestimide did not increase the serum TG concentrations and significantly decreased serum LDL-C concentrations in FH

patients with hyper- and with normotriglyceridemia. The conventional bile-acid-sequestering resin (cholestyramine) is known to potentially increase TG-rich lipoproteins?[29] Although there are no data directly comparing the concentrations of TG after treatment with cholestyramine or colestimide, Homma et al reported that colestimide monotherapy decrease plasma LDL-C concentrations (14.2%) without affecting plasma TG, very-low-density lipoprotein cholesterol and very-low-density lipoprotein triglyceride concentrations?[30] In an animal study, cholestyramine increased the ratio between 3-hydroxyl-3-methylglutaryl coenzyme A reductase and cholesterol-7-α-hydroxylase activity (1.7–2.0), which led to hypertriglyceridemia because of overproduction of very-low-density lipoprotein?[31] In contrast, colestimide did not increase that ration (0.8–1.3)?[31]

A potential limitation of the current study is that the study patients took different doses of atorvastatin (between 20 and 40 mg): 7 (47%) FH patients took 40 mg of atorvastatin, which is the maximum permitted dose in Japan. Ten FH patients (67%) already had a history of CAD, so they needed intensive treatment with higher doses of atorvastatin. On the other hand, 5 FH patients who were free from CAD (according to the Japan Atherosclerosis Society's guidelines; their target LDL-C concentrations were 140–120 mg/dl) had lower doses of atorvastatin. Because the focus of the current study was the additional efficacy of combining colestimide with atorvastatin, we believe that the different doses of atorvastatin did not confound the results.

In the current study, FH was diagnosed by our clinical criterion[2,18,19] and confirmed by genetic method in 14 of the 15 enrolled patients![8,19] We failed to find the LDL receptor gene mutation in 1 patient, whose baseline LDL-C concentration was almost the average for heterozygous FH, namely 262 mg/dl. Because no patient with familial defective apolipoprotein B has been reported in Japan?[32] it is possible that perhaps this particular patient might be another case of autosomal dominant hypercholesterolemia, such as PCSK9 gene mutation?[33]

In conclusion, we found that colestimide produces an additional 20% reduction of serum LDL-C concentration when administered with atorvastatin. Of note, colestimide did not increase serum TG concentrations even in heterozygous FH with hypertriglyceridemia. Thus, we conclude that when monotherapy with atorvastatin is insufficient to treat severely hypercholesterolemic patients, LDL-C can be further reduced by the coadministration of colestimide.

Acknowledgments

We express our special thanks to Mayumi Yoshida, Mihoko Mizuno, and Sachio Yamamoto for their outstanding technical assistance.

References

1. Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. *In*: Scriver CR, Beaudet AL, Sly WS, Valle I, editors. The metabolic basis of inheritated disease, 8th edn. New York: McGrawHill; 2001; 2863–2913.
2. Mabuchi H, Koizumi J, Shimizu M, Takeda R. Development of coronary heart disease in familial hypercholesterolemia. *Circulation* 1989; **79:** 225–232.
3. Imamura H, Izawa A, Kai R, Yokoseki O, Uchikawa S, Yazaki Y, et al. Trends over the last 20 years in the clinical background of young Japanese patients with coronary artery disease. *Circ J* 2004; **68:** 186–191.
4. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; **344:** 1383–1389.
5. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995; **333:** 1301–1307.
6. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels: Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996; **335:** 1001–1009.
7. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: Results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 1998; **279:** 1615–1622.
8. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels: The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. *N Engl J Med* 1998; **339:** 1349–1357.
9. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: A randomised placebo-controlled trial. *Lancet* 2002; **360:** 7–22.
10. Mortality in treated heterozygous familial hypercholesterolaemia: Implications for clinical management. *Atherosclerosis* 1999; **142:** 105–112.
11. Mabuchi H, Haba T, Tatami R, Miyamoto S, Sakai Y, Wakasugi T, et al. Effects of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoproteins and ubiquinone-10-levels in patients with familial hypercholesterolemia. *N Engl J Med* 1981; **305:** 478–482.
12. Hata Y, Mabuchi H, Saito Y, Itakura H, Egusa G, Ito H, et al. Working Committee on JAS Guideline for Diagnosis and Treatment of Hyperlipidemias: Report of the Japan Atherosclerosis Society (JAS) Guideline for Diagnosis and Treatment of Hyperlipidemia in Japanese adults. *J Atheroscler Thromb* 2002; **9:** 1–27.
13. Prevention of coronary heart disease in clinical practice: Recommendations of the Second Task Force of European and other Societies on Coronary Prevention. *Eur Heart J* 1998; **19:** 1434–1503.
14. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285:** 2486–2497.
15. Mabuchi H, Sakai T, Sakai Y, Yoshimura A, Watanabe A, Wakasugi T, et al. Reduction of serum cholesterol in heterozygous patients with familial hypercholesterolemia: Additive effects of compactin and cholestyramine. *N Engl J Med* 1983; **308:** 609–613.
16. Shepherd J, Packard CJ, Bicker S, Lawrie TD, Morgan HG. Effect of cholestyramine on low-density lipoproteins. *N Engl J Med* 1980; **303:** 943–944.
17. Mitsubishi Chemical Corporation. MCI-196: Bile acid sequestrant. *Drugs Future* 1993; **18:** 15.
18. Mabuchi H, Ito S, Haba T, Ueda K, Ueda R. Discrimination of familial hypercholesterolemia and secondary hypercholesterolemia by Achilles' tendon thickness. *Atherosclerosis* 1977; **28:** 61–68.
19. Yagi K, Hifumi S, Nohara A, Higashikata T, Inazu A, Mizuno K, et al. Difference in the risk factors for coronary, renal and other peripheral arteriosclerosis in heterozygous familial hypercholesterolemia. *Circ J* 2004; **68:** 623–627.
20. Yu W, Nohara A, Higashikata T, Lu H, Inazu A, Mabuchi H. Molecular genetic analysis of familial hypercholesterolemia: Spectrum and regional difference of LDL receptor gene mutations in Japanese population. *Atherosclerosis* 2002; **165:** 335–342.
21. Arranz-Pena ML, Tasende-Mata J, Martin-Gil FJ. Comparison of two homogeneous assays with a precipitation method and an ultracentrifugation method for the measurement of HDL-cholesterol. *Clin Chem* 1998; **44:** 2499–2505.
22. Nakamura M, Taniguchi Y, Yamamoto M, Hino K, Manabe M. Homogeneous assay of serum LDL-cholesterol on an automatic analyzer. *Clin Chem* 1997; **43:** S260.
23. Kajinami K, Mabuchi H, Koizumi J, Takeda R. Serum apolipoproteins in heterozygous familial hypercholesterolemia. *Clin Chim Acta* 1992; **211:** 93–99.
24. Hunninghake D, Insull W Jr, Toth P, Davidson D, Donovan JM, Burke SK. Coadministration of colesevelam hydrochloride with atorvastatin lowers LDL-C additively. *Atherosclerosis* 2001; **158:** 407–416.
25. Heinonen TM, Schrott H, McKenney JM, Sniderman AD, Broyles FE, Zavoral JH, et al. Atorvastatin, a new HMG-CoA reductase inhibitor as monotherapy and combined with colestipol. *J Cardiovasc*

Pharmacol Ther 1996; **1:** 117–122.
26. Kiortisis DN, Millionis H, Bairaktari E, Elisaf MS. Efficacy of combination of atorvastatin and micronised fenofibrate in the treatment of severe mixed hyperlipidemia. *Eur J Clin Pharmacol* 2000; **56:** 631–635.
27. Eriksson M, Hadell K, Holme I, Walldius G, Kjellstrom T. Compliance with and efficacy of treatment with pravastatin and cholestyramine: A randomized study on lipid-lowering in primary care. *J Intern Med* 1998; **243:** 373–280.
28. Mabuchi H, Koizumi J, Shimizu M, Kajinami K, Miyamoto S, Ueda K, et al. Long-term efficacy of low-density lipoprotein apheresis on coronary heart disease in familial hypercholesterolemia: Hokuriku-FH-LDL-Apheresis Study Group. *Am J Cardiol* 1998; **82:** 1489–1495.
29. Brensike JF, Levy RI, Kelsey SF, Passamani ER, Richardson JM, Loh IK, et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: Results of the NHLBI Type II Coronary Intervention Study. *Circulation* 1984; **69:** 313–324.
30. Homma Y, Kobayashi T, Yamaguchi H, Ozawa H, Sakane H, Nakamura H. Specific reduction of plasma large, light low-density lipoprotein by a bile acid sequestering resin, cholebine (MCI-196) in type II hyperlipoproteinemia. *Atherosclerosis* 1997; **129:** 241–248.
31. Suzuki K, Kaneko N, Nemoto A, Shimada H. Difference between cholestyramine and colestimide (colestilan), a novel hypolipidemic resin concerning regulation of hepatic cholesterol metabolism (abstract). *Atherosclerosis* 2000; **151:** 135.
32. Nohara A, Yagi K, Inazu A, Kajinami K, Koizumi J, Mabuchi H. Absence of familial defective apolipoprotein B-100 in Japanese patients with familial hypercholesterolaemia. *Lancet* 1995; **345:** 1438.
33. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34:** 154–156.