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Serum Lp(a) concentrations are unaffected by treatment with the HMG-CoA reductase inhibitor Pravastatin: results of a 2-year investigation

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Summary

The concentration of lipoprotein (a) in plasma is under stringent genetic control and raised concentrations are strongly linked to coronary heart disease, in particular when low density lipoprotein levels are also increased. We serially monitored serum Lp(a) in 14 hypercholesterolemic patients who were treated with Pravastatin over a period of two years. C-reactive protein levels were also quantified to exclude a possible 'acute-phase' response as a reason for a sudden increase in the Lp(a) concentration. No significant changes were seen in mean Lp(a) levels after 24 months of therapy. Considerable fluctuations of serum Lp(a) levels occurred during the course of treatment. These were in some cases associated with raised C-reactive protein concentrations and might therefore be attributable to an 'acute-phase' response. We conclude that the HMG-CoA reductase inhibitor Pravastatin has no long-lasting effects on Lp(a) levels in hypercholesterolemic patients suffering from coronary heart disease.

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Introduction

The lipoprotein Lp(a) is similar to LDL in composition and structural organisation, but is distinguished from it by the presence of a high molecular mass glycoprotein, apo(a), which is highly homologous to the plasma protease zymogen plasminogen [1, 2]. The concentration of Lp(a) in plasma is under stringent genetic control, increased concentrations being strongly linked to premature cardiovascular disease [3], myocardial infarction [4] and cerebrovascular disease [5]. The plasma concentrations of this lipoprotein in plasma are generally refractory to conventional lipid-lowering therapies. Low-fat or high-polyunsaturated fat diets are ineffective in reducing the levels of this lipoprotein [6] as are bile acid resins [7], and fibric acid derivatives [8]. Moderate reductions in Lp(a) concentrations have been reported with nicotinic acid derivatives [9, 10]. There have been conflicting reports on the effect of the mild reducing agent *N*-acetylcysteine on Lp(a) concentrations [11, 12]. The anabolic steroid stanozolol has been shown to reduce Lp(a) levels by up to 80% [13]. Contrasting reports have been published concerning the effects of HMG-CoA reductase inhibitors on Lp(a). Whereas some authors [14–16] have observed no alterations in Lp(a) levels under this cholesterol-lowering therapy, others [17, 18] reported a significant increase in Lp(a) concentrations on treatment with Lovastatin or Simvastatin. These studies, however, only covered short-term treatment with these drugs.

We have now followed serum Lp(a) concentrations in 14 patients with angiographically assessed CHD (coronary heart disease) treated with Pravastatin over a period of 2 years. Since Lp(a) levels have been shown to increase during the 'acute-phase' response [19] we also quantified C-reactive protein (CRP) in order to exclude a possible 'acute-phase' response as a reason for an increase in the Lp(a) concentration.

Materials and methods

Study protocol

Fourteen patients, suffering from CHD and primary hypercholesterolemia (12 males, 2 females; aged 36–66 years) were treated with the HMG-CoA reductase inhibitor Pravastatin (Squibb/von Heyden, Princeton NJ, USA) over a period of 2 years. After a 4-week placebo, treatment was started with 20 mg/day for the first 2 months and increased to 40 mg/day for the remaining 22 months. Blood samples were drawn every 2 months for evaluation of chemical and hematological variables. The study protocol was approved by the local university ethics committee.

Methods

Blood samples for measurement of Lp(a) were collected at 0, 2, 4, 8, 12, 18, and 24 months. The blood was allowed to clot and was centrifuged at 1,000 × *g* for 10

min at room temperature. The supernatant serum was stored at −20°C until assay. Lp(a) concentrations were quantified using a specific polyclonal ELISA [20]; all samples from a single patient were assayed in duplicate on the same microtitre plate to avoid interassay variation. Cross-reactivity with human plasminogen was <0.02% with this assay. The intraassay-variation of the ELISA was 10%. Apoprotein B and CRP concentrations were determined by immunologic-nephelometric methods (Beckmann Array Protein Sys., Beckman Instruments Inc., Brea, CA, USA). CRP-concentrations above 100 mg/l were considered to be abnormal. Total cholesterol and total triglycerides were determined using enzymatic test-kits (Boehringer Mannheim, Mannheim, FRG) while LDL-cholesterol (Immuno GmbH, Heidelberg, FRG) and HDL-cholesterol (Boehringer Mannheim, Mannheim, FRG) were measured by precipitation techniques. Apo E-phenotypes were determined by isoelectric focussing of the plasma apo VLDL-fraction after isolation by ultracentrifugation and delipidation [21].

Statistical methods

The Wilcoxon-paired test was used to compare baseline values of Lp(a) with the values obtained after 24 months.

In order to determine whether a significant change in Lp(a) concentrations occurred during the course of Pravastatin treatment within a particular individual the method of critical differences was employed [22]. The critical difference is used in the longitudinal assessment of analytical values in the same individual over a period of time and is derived as follows. If 2 random variables are independent and have the same expected value and the same standard deviation δ then the standard deviation of their difference will be $\sqrt{2}\delta$. The probability that the absolute value of the difference is less than or equal to $2\sqrt{2}\delta$ is about 95% assuming gaussian distribution. Knowing the standard deviation of the assay system, in this case the ELISA for Lp(a), the absolute value of the difference between 2 independent measurements can then be calculated and if this is greater than the critical difference, then the results can be regarded as significantly different with regard to the analytical test used.

Results

Baseline clinical and biochemical data of the patients with angiographically assessed CHD who participated in this study are shown in Table I. Patients 9 and 10 had the heterozygous form of FH as suggested by LDL-receptor activities below 60% compared to normolipidemic healthy controls. Serum total cholesterol and LDL-cholesterol concentrations ranged from 6.15 to 17.84 mmol/l and from 4.12 to 13.81 mmol/l, respectively, prior to beginning therapy with Pravastatin. Treatment of the 14 patients with 20 mg/day of the HMG-CoA reductase inhibitor Pravastatin led to a significant reduction in mean total cholesterol (−15%, $P<0.005$), LDL-

TABLE I

Baseline characteristics of the patients

No. Pat.	Sex (M/F)	LP(a) (g/l)	Chol (mmol/l)	TG (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	Apo E Isof.
1	M	0.048	7.01	1.93	4.78	1.16	3/2
2	M	0.057	7.34	1.15	5.79	1.37	3/3
3	M	0.396	6.65	3.84	4.6	0.52	3/3
4	M	0.102	6.15	1.36	4.81	0.83	3/3
5	F	0.12	17.84	1.81	13.81	1.71	3/3
6	M	0.204	9.93	2.62	7.73	1.53	3/3
7	M	0.162	10.09	1.05	8.59	1.5	4/3
8	M	0.201	7.73	2.36	5.28	0.83	3/3
9	M	0.198	8.51	1.74	6.18	1.47	3/3
10	M	0.6	9.03	0.98	7.11	1.71	3/3
11	M	0.03	8.77	2.23	6.67	1.68	3/3
12	M	0.162	6.62	1.45	5.06	1.03	3/3
13	M	0.4	6.15	2.15	4.42	0.88	3/3
14	F	0.306	6.72	1.65	5.48	1.4	3/3

cholesterol (-27%, $P<0.005$) and a slight but not significant reduction in triglyceride values (-5%, $P>0.05$) (Fig. 1). On increasing the dose to 40 mg/day, a further decrease in the levels of these parameters was observed which remained relatively stable

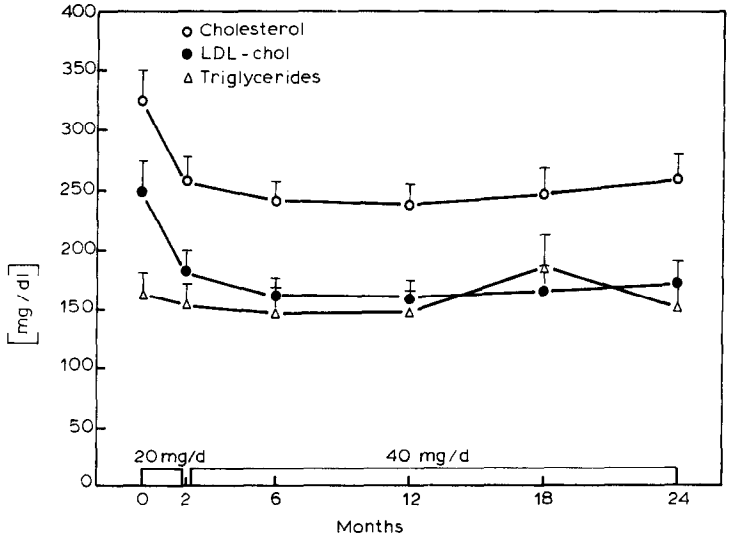

Fig. 1. Mean total cholesterol, LDL-cholesterol, and triglycerides levels during a 2-year treatment with Pravastatin in 14 patients.

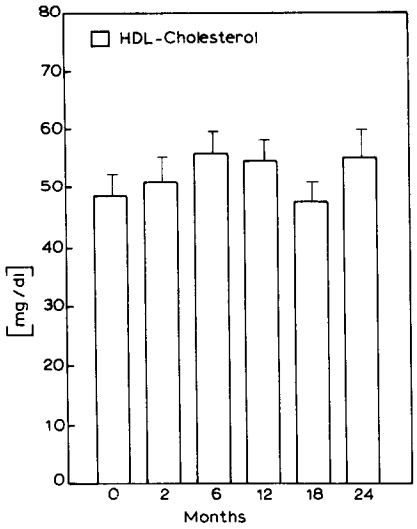

Fig. 2. Mean HDL-cholesterol levels during a 2-year treatment with Pravastatin in 14 patients.

thereafter for the course of the study (total-chol: −24%, $P<0.005$; LDL-chol: −32%, $P<0.005$; triglycerides: −7%, $P>0.05$) (Fig. 1). Mean HDL-cholesterol levels showed a general increase during the 24-month study period (+13%, $P>0.05$;) (Fig. 2). The decrease in total cholesterol and LDL-cholesterol was accompanied by a significant reduction in apoprotein B 100 after 12 months of treatment (−26%, $P<0.005$) (Fig. 3). No significant changes were seen in mean Lp(a) levels after 24

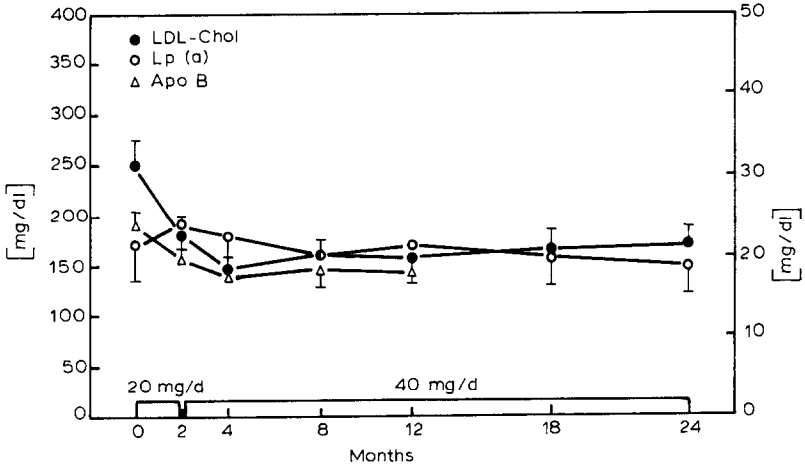

Fig. 3. Mean LDL-cholesterol, apolipoprotein B, and Lp(a) levels during a 2-year treatment with Pravastatin in 14 patients.

months of therapy when compared with baseline values (Fig. 3, Table II). However, after 2 months of treatment a slight, but not significant increase in mean Lp(a) levels was observed (Fig. 3) as compared to baseline, mainly due to an increase in the Lp(a) concentrations of 7 patients (Table III). Since Lp(a) has been shown to increase during the 'acute phase' response, we evaluated whether these increases could be explained by this phenomenon. After 2 months of therapy only 1 of these 7 patients had a coincident increase in Lp(a) and CRP. During the remainder of the study we calculated 12 significant elevations of Lp(a) concentrations by the method of critical difference. Eight of these values were accompanied by an increase in CRP-levels (Table III). These events might therefore be explained by 'acute-phase' responses. One patient (No. 12) had high levels of both Lp(a) and CRP at the onset of therapy. Thereafter both parameters decreased (Table III). In this case another 'acute-phase' response can be suspected at the start of the treatment. After 24 months 6 patients had significantly lower Lp(a) levels than at the beginning of the study as calculated by the method of critical difference. They did not have signs of an 'acute-phase' response at baseline as documented by normal CRP levels. In 6 patients the Lp(a) level did not change significantly during 24 months of treatment with 40 mg/day Pravastatin. In only 2 patients were the Lp(a) levels elevated after 24 months as compared to base-

TABLE II

Effectiveness of LDL-cholesterol lowering after 2 and 24 months therapy with Pravastatin and relationship to the corresponding Lp(a) concentrations

No.	Baseline			20 mg/day			40 mg/day	
Pat.	LDL-Chol (mmol/l)	Lp(a) (g/l)	LDL-Chol reduction (%)	LDL-Chol. (mmol/l)	Lp(a) (g/l)	LDL-Chol. reduction (%)	LDL-Chol. (mmol/l)	Lp(a) (g/l)
1	4.48	0.048	−36	3.05	0.086	−15	4,09	0.052
2	5.79	0.057	−33	3.9	0.124	−47	3.05	0.062
3	4.6	0.396	−25	3.47	0.41	−12	4.09	0.345
4	4.81	0.102	−10	4.34	0.068	−30	3.36	0.054
5	13.81	0.12	−25	10.37	0.015	−27	10.09	0.072
6	7.73	0.204	−39	4.76	0.245	−30	5.4	0.21
7	8.59	0.162	−37	5.46	0.23	−39	5.22	0.196
8	5.28	0.201	−11	4.71	0.24	−20	4.24	0.23
9	6.18	0.198	−38	3.83	0.188	−40	3.7	0.15
10	7.11	0.6	−20	5.66	0.59	−56	3.05	0.396
11	6.67	0.03	−36	4.29	0.028	−48	3.44	0.017
12	5.04	0.163	−16	4.24	0.155	− 8	4.68	0.128
13	4.42	0.4	−17	3.67	0.4	−19	3.59	0.39
14	5.48	0.306	−39	3.7	0.44	−29	3.88	0.315
Mean	6.44	0.21		4.68	0.24		4.43	0.19
±SD	2.46	0.16		1.78	0.16		1.78	0.13

TABLE III

Lp(a) concentrations (g/l) during Pravastatin treatment

	Time (months)						
	0	2	4	8	12	18	24
	Dose (mg/l)						
No. Pat.	0	20	40	40	40	40	40
1	0.048	0.086*	0.048	0.126**	0.047	0.075*	0.052
2	0.057	0.124*	0.084**	0.077**	0.062	0.069**	0.062
3	0.396	0.41	0.43	0.342	0.401	0.342	0.342
4	0.102	0.068	0.129*	0.056	0.068	0.059	0.054
5	0.12	0.15*	0.135	0.117	0.104	0.12	0.072
6	0.204	0.245*	0.2	0.186	0.201	0.216	0.21
7	0.162	0.23*	0.29*	0.177	0.174	0.195**	0.196*
8	0.201	0.24**	0.22	0.24**	0.228	0.219	0.23**
9	0.198	0.188	0.197	0.153	0.186	0.192	0.15
10	0.6	0.59	0.54	0.504	0.588	0.492	0.396
11	0.03	0.028	0.025	0.029	0.033	0.033	0.017
12	0.162**	0.155	0.13	0.099	0.12	0.12	0.128
13	0.4	0.4	0.42	0.441	0.473**	0.378	0.39
14	0.306	0.44*	0.308	0.257	0.27	0.261	0.315

*, Significant elevation without increase in CRP level.

**, Significant elevation with a coincident increase in CRP level.

line. Finally, the Lp(a) concentration appeared to have no effect on the effectiveness of LDL-cholesterol reduction by Pravastatin (Table II).

Discussion

The role of hypercholesterolemia as a factor in the development of coronary heart disease (CHD) is firmly established [23]. Elevated LDL-cholesterol levels in particular are closely related to the incidence of cardiovascular diseases [24]. Lp(a) levels of 20–30 mg/dl and above are considered to be an independent risk factor for the development of CHD [25]. When LDL-cholesterol and Lp(a) levels are both elevated the relative risk for CHD is raised to 5- or 6-fold [3]. The HMG-CoA reductase inhibitors have proven to be extremely potent drugs for lowering elevated cholesterol concentrations as confirmed in the present investigation (Fig. 1). However, there are conflicting reports as to the effect of these drugs on Lp(a). An investigation conducted by Thiery et al. revealed no changes in Lp(a) levels after treatment with Simvastatin over a period of 3 months [15]. Berg and Leren reported unchanged serum Lp(a) concentrations on treatment with up to 80 mg/day Lovastatin over 3 months [14], and Jacob et al. reported similar findings with the HMG-CoA reductase inhibi-

tors Lovastatin and Pravastatin over a period of 4 months [16]. In contrast Jürgens et al. observed variable increases in Lp(a) in 8 patients during Lovastatin therapy over a period of 3 months [18], while Kostner et al. found a significant increase in mean Lp(a) levels after therapy with Simvastatin or Lovastatin [17]. We have now followed 14 patients treated with Pravastatin over a much longer period of time (24 months) and found a considerable fluctuation of serum Lp(a) levels during the course of treatment. In the present study 50% of the significant increases in serum Lp(a) levels within an individual could be attributed to an 'acute-phase' response as judged by concomitantly raised CRP-levels. However, the mean Lp(a) values remained constant for the treated group after 24 months. As observed by Jürgens et al. and Kostner et al. mean Lp(a) concentrations rose at the beginning of therapy in several patients (Fig. 3). We cannot explain this phenomenon but disturbances in lipid metabolism as proposed by these authors cannot be excluded. However, the observed increases were of a transient nature and had generally returned to basal values at the next examination. In our study the observed changes in Lp(a) cannot be explained by analytical problems since we collected and stored serum samples at $-20°C$ until assay. All samples from a single patient were measured in duplicate on the same microtitre plate to avoid interassay variation. From the previously published studies the protocol for Lp(a) measurements is not obvious. Although not all significant elevations in Lp(a), as estimated from the critical difference [22], were associated with a raised CRP-level it should be noted that CRP is a rapidly responding acute-phase protein which quickly returns to normal values on remission. Lp(a) on the other hand is a much slower acute-phase reactant, reaching maximum levels 6–10 days after tissue damage [19]. Not all 'acute-phase' responses may, therefore, have been detected using the CRP measurements.

We conclude that the HMG-CoA reductase inhibitor Pravastatin has no long-lasting effects on Lp(a) levels in hypercholesterolemic patients suffering from CHD. If one measures elevated concentrations of Lp(a), the assessment of a possible 'acute-phase' response may be useful for interpretation of the Lp(a) concentration. Further investigation into the biologic variation of Lp(a) levels in individuals not under treatment with HMG-CoA reductase inhibitors would appear to be desirable.

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