Tokai J Exp Clin Med., Vol. 30, No. 1, pp. 63-69, 2005

Effect of anticholesterol therapy on soluble ICAM-1 in chronic stroke patients with hyperlipidemia

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(Received November 17, 2004; Accepted December 13, 2004)

Objective: We examined the effects of drug therapy with pravastatin (P) or bezafibrate (B) and diet (D) therapy on serum lipids and soluble intercellular adhesion molecule-1 (sICAM-1) in hyperlipidemic cerebrovascular disease (CVD) patients in the chronic stage.

Methods: This study included 36 patients (28 with cerebral infarction and hyperlipidemia and eight with cerebral hemorrhage and hyperlipidemia) divided into three groups: Group P (12 patients), Group B (10 patients), and Group D (14 patients). Before and after treatment, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and sICAM-1 levels were measured.

Results: In Group P, Group B and Group D, TC levels were decreased by 30% ($p < 0.005$), 21% ($p < 0.01$), and 21% ($p < 0.001$), LDL-C levels were decreased by 38% ($p < 0.005$), 18% (not significant), and 25% ($p < 0.005$) and TG levels were decreased by 27% ($p < 0.05$), 53% ($p < 0.005$) and 22% ($p < 0.05$), respectively. sICAM-1 levels were decreased by 20% ($p < 0.005$) in Group P, but were not decreased in Group B or Group D. There was no correlation between ΔTC and ΔsICAM-1 ($r = 0.172$).

Conclusion: Administration of pravastatin significantly reduced sICAM-1 levels, independently of its decreasing effect on TC and TG in chronic CVD patients. Pravastatin may exert anti-atherosclerotic activity via two distinct mechanisms.

Key words : intercellular adhesion molecule-1 (ICAM-1), ischemic stroke, pravastatin, hyperlipidemia

INTRODUCTION

Intercellular adhesion molecule-1 (ICAM-1) is a type N sugar chain-binding glycoprotein with a molecular weight of 75 to 115 kDa, belonging to the immunoglobulin superfamily [16]. Leukocytes in circulating blood initially decelerate on the endothelial cell surface by rolling via selectin, which is induced on the endothelial cell surface by stimuli such as thrombin. They are then strongly bound to endothelial cells through ICAM-1 on the endothelial cell surface and $\beta 2$ integrins, such as leukocyte function-associated molecule-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) on the leukocyte surface [1]. Subsequent invasion of these leukocytes into the vascular media is considered to be involved in the onset of arteriosclerosis [3].

In this study, we examined the influence of drug therapy and diet therapy on serum levels of soluble ICAM-1 (sICAM-1) in hyperlipidemic patients with chronic-stage cerebrovascular disease.

MATERIALS AND METHODS

The study included 28 patients with cerebral infarction in the chronic stage (30 days or more after the onset of CVD) associated with hyperlipidemia, and 8 patients with cerebral hemorrhage in the chronic stage and

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Table 1 Characteristics of patients

	sex		age	intervals (months)	treatment period (days)
	male	female	(mean±SD)	(mean±SD)	(mean±SD)
pravastatin (n=12)	3	9	69±9	2.8±1.7	43±26
bezafibrate (n=10)	6	4	66±8	4.3±3.0	25±10
diet (n=14)	3	11	70±12	3.2±1.2	54±28

ns: pravastatin vs bezafibrate; pravastatin vs diet; bezafibrate vs diet (age, intervals)
ns: pravastatin vs bezafibrate; bezafibrate vs diet (treatment period)
*: pravastatin vs diet (treatment period)

* $p < 0.005$

hyperlipidemia. After informed consent for enrollment in this study had been obtained, these patients were divided into three groups: Group P treated with 10 mg/day of pravastatin sodium (n = 12, three men, nine women, age: 69 ± 9 years, administration period: 43 ± 26 days), Group B treated with 200 mg/day of bezafibrate (n = 10, six men, four women, age: 66 ± 8 years, administration period: 25 ± 10 days), and Group D treated by diet therapy alone (n = 14, three men, 11 women, age: 70 ± 12 years, treatment period: 54 ± 28 days). The proportion of women was higher in Groups P and D, but there were no significant differences in mean age among the three groups. Mean intervals between the onset of cerebrovascular disease and the commencement of treatment were 2.8 ± 1.7 months in Group P, 4.2 ± 3.0 months in Group B, and 3.2 ± 1.2 months in Group D. There were no significant differences of age, interval or treatment period among the three groups (Table 1). According to the Guideline for diagnosis and treatment of hyperlipidemias in adults published by the Japan Atherosclerosis Society [8], the criteria for hyperlipidemia used in this study included a total cholesterol (TC) level of 200 mg/dl or more, a low density lipoprotein cholesterol (LDL-C) level of 120 mg/dl or more in patients without coronary disease as well as a TC level of 180 mg/dl or more, or an LDL-C level of 100 mg/dl or more in patients with coronary disease. In all three groups, diet therapy was administered: daily energy intake, 1400-1600 kcal (25 kcal/kg); protein, 70-75 g; lipids, 40-45 g; and saccharides, 190-220 g. Fasting serum sICAM-1, TC, high density lipoprotein cholesterol (HDL-C), triglyceride (TG), and LDL-C levels were measured before and after treatment. sICAM-1 levels were measured by two-step solid phase sandwich enzyme-linked immunosorbent assay using a kit (Bender Medsystems Inc.). In this procedure, an anti-ICAM-1 monoclonal antibody labeled with horseradish peroxidase was used as a solid phase antibody. Since inflammation increases ICAM-1 levels, patients with C reactive protein levels of 0.3 mg/dl or more were excluded from this study. For statistical analysis, Wilcoxon's coded rank test was used.

RESULTS

(1) Total cholesterol (Table 2)

In Group P, the mean TC level after treatment was significantly decreased from 280 ±52 to 197±39 mg/dl (30% reduction, $p < 0.005$). In Group B, the mean TC level after treatment was significantly decreased from 241 ± 39 to 191 ± 23 mg/dl (21% reduction, $p < 0.01$). In Group D, the mean TC level after treatment was significantly decreased from 255 ± 37 to 201 ± 32 mg/dl (21% reduction, $p < 0.001$). There were no significant differences in pretreatment TC levels among Groups P, D, and B.

(2) LDL cholesterol (Table 2)

In Group P, the mean LDL-C level was significantly decreased from 192 ± 30 to 120 ± 26 mg/dl (38% reduction, $p < 0.005$). In Group B, the mean LDL-C level was decreased, but not significantly, from 149 ± 33 to 122 ± 28 mg/dl (18% reduction). In Group D, the mean LDL-C level was significantly decreased from 169 ± 36 to 127 ±

Table 2 Fasting serum sICAM-1, TC, LDL-C, TG and HDL-C levels before and after treatment.

		sICAM-1 (ng/ml)	TC (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)
pravastatin	before	397±72 *	280±52 *	192±30 *	170±51 §	58±18
	after	319±63	197±39	120±26 *	124±61	56±17 §
bezafibrate	before	356±72 *	241±39 †	149±33	255±151 †	42±11
	after	340±65	191±23	122±28	121±41 §	45±11 §
diet	before	310±71	255±37 ‡	169±36 *	148±70 §	57±17
	after	321±81	201±32	127±28	115±38	51±9

* $p < 0.005$; † $p < 0.01$; ‡ $p < 0.001$; § $p < 0.05$

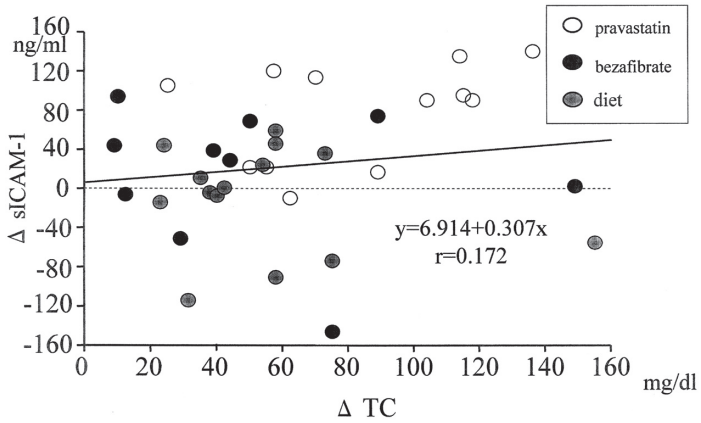

Fig. 1 Relationship between Δ TC and Δ ICAM-1 in all patients

28 mg/dl (25% reduction, $p < 0.005$). There was a significant difference ($p < 0.005$) in pretreatment LDL-C levels between Group P and Group B.

(3) Triglyceride (Table 2)

In Group P, the mean TG level was significantly decreased from 170 ± 51 to 124 ± 61 mg/dl (27% reduction, $p < 0.05$). In Group B, the mean TG level was significantly decreased from 255 ± 151 to 121 ± 41 mg/dl (53% reduction, $p < 0.01$). In Group D, the mean TG level was significantly decreased from 148 ± 70 to 115 ± 38 mg/dl (23% reduction, $p < 0.05$). There was a significant difference ($p < 0.05$) in pretreatment TG levels between Group B and Group D.

(4) HDL cholesterol (Table 2)

There were no significant changes in the mean HDL-C level in any group, although there were significant differences in pretreatment HDL-C levels between Group P and Group B ($p < 0.05$), as well as between Group B and Group D ($p < 0.05$).

(5) sICAM-1 (Table 2)

In Group P, the mean sICAM-1 level (normal value: 224 ± 41 ng/ml) was significantly decreased from 397 ± 72 to 319 ± 63 ng/ml (20% reduction, $p < 0.005$). In Group B and Group D, there was no significant change in sICAM-1 levels. However, there was a significant difference ($p < 0.005$) in pretreatment sICAM-1 levels between Group P and Group D.

(6) Relationships of ΔTC, ΔLDL-C, ΔTG, and ΔHDL-C to ΔICAM-1 in all patients (Figs. 1-4)

Overall, changes in sICAM-1 levels (Δ sICAM-1) were examined in relation to changes in TC, LDL-C, TG, and HDL-C levels after treatment. However, there were no

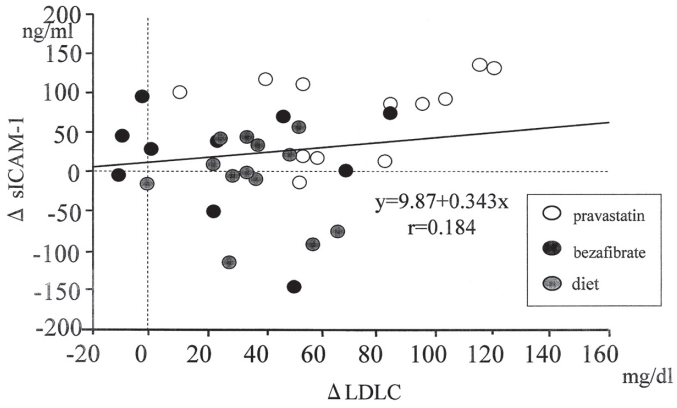

Fig. 2 Relationship between Δ LDLC and ΔICAM-1 in all patients

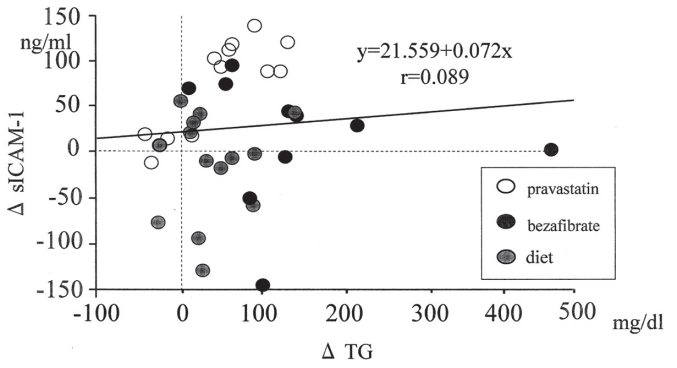

Fig. 3 Relationship between Δ TG and ΔICAM-1 in all patients

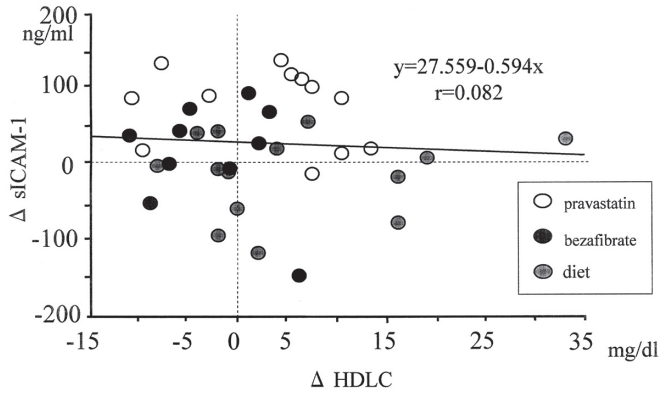

Fig. 4 Relationship between ΔHDLC and ΔICAM-1 in all patients

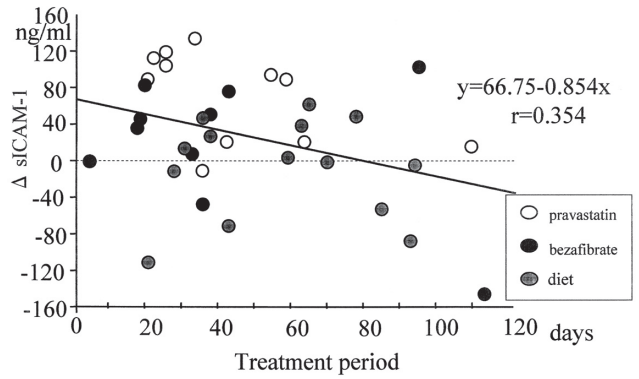

Fig. 5 Relationship between treatment period and Δ ICAM-1 in all patients

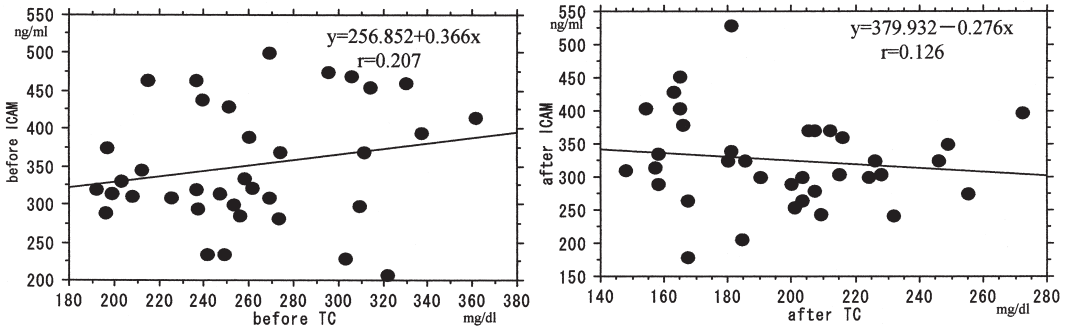

Fig. 6 Relationship between TC and sICAM-1 before and after treatment in all patients

correlations between these changes (ΔTC, r = 0.172; ΔLDL-C, r = 0.184; ΔTG, r = 0.089; ΔHDL-C, r = 0.082).

(7) Relationship between treatment duration and ΔICAM-1 in all patients (Fig. 5)

There was no correlation between the treatment duration and ΔsICAM-1 in all patients (r = 0.354).

(8) Relationship between TC and sICAM-1 in all patients (Fig. 6)

There was no correlation between TC and sICAM-1 before (r = 0.207) and after (r = 0.126) treatment in all patients.

DISCUSSION

In this study, we examined the effects of various anti-cholesterol therapies on serum lipid and sICAM-1 levels in hyperlipidemic CVD patients in the chronic stage. These therapies all reduced TC levels, but only pravastatin significantly reduced sICAM-1 levels. Previous cerebral ischemia-reperfusion experiments have demonstrated ICAM-1 expression in endothelial cells in the acute stage [11]. Some clinical studies have indicated that sICAM-1 levels are increased in the subacute and chronic stages of CVD [17]. Clark *et al.* [2] and Zhang *et al.* [19, 20] reported that administration of an anti-ICAM-1 antibody improved ischemic cerebral cellular dysfunction in rabbit and rat middle cerebral artery occlusion models. Furthermore, Kitagawa *et al.* [9] examined the influence of ICAM-1 on lesion size, granular accumulation, and microcirculatory disorder in the presence of local cerebral ischemia using ICAM-1-knockout mice, and reported that ICAM-1 did not influence granular accumulation, but induced microcirculatory disorder, causing extension of cerebral infarction. Thus, inhibition of ICAM-1 expression or administration of an anti-ICAM-1 antibody might have a beneficial effect in cerebral infarction. Ridker *et al.* [12] indicated that raised concentration of sICAM-1 was a risk factor for myocardial infarction in healthy men. Furthermore, Hwang *et al.* [7] reported that sICAM-1 and selectin were independent risk factors for coronary heart disease and carotid arteriosclerosis. These findings suggest that sICAM-1 levels increase not only after cerebral ischemia, but also prior to stroke, so that sICAM-1 may be an important molecular marker of arteriosclerosis. We previously reported that not only patients with cerebral infarction in the chronic stage, but also apparently healthy subjects with risk factors for cerebrovascular disease showed significantly higher sICAM-1 levels than healthy adults [6]. This finding suggests that increases in sICAM-1 were not simply induced by cerebrovascular attacks themselves, and that sICAM-1 may be an indicator for the presence of latent vascular lesions.

With respect to the relationship between ICAM-1 and serum sICAM-1, Rosenstein *et al.* [13] reported a positive correlation between ICAM-1 levels on the endothelial cell surface and serum sICAM-1 levels. Therefore, serum sICAM-1 levels may reflect ICAM-1 levels on the cell surface.

In this study, we examined the influence of drug (pravastatin and bezafibrate) or diet therapy on sICAM-1 in hyperlipidemic patients with cerebrovascular disease in the chronic stage. In Groups P, B, and D, TC levels were significantly decreased, suggesting that diet therapy and drug therapy improved hyperlipidemia. However, the sICAM-1 level was significantly decreased only in Group

P. There are two issues here. One is that the mean treatment period in Group B (25 ± 10 days) was shorter than those in two other groups (there was no significant difference between Group B and Group P, but there was a significant difference between Group B and Group D). The other is that the mean pretreatment sICAM-1 level in Group P was higher than those in the two other groups (there was no significant difference between Group P and Group B, but there was a significant difference between Group P and Group D). We considered that the former may not have influenced the results of this study, because there was no correlation between the treatment period and ΔsICAM-1, as shown in Fig. 5. The latter may also have had no influence, because even in Group D, the mean sICAM-1 level showed abnormal change with a significant difference ($p < 0.01$) from the normal value, and because there was no correlation between ΔTC and ΔsICAM-1, and between TC and sICAM-1 before and after treatment in all patients. Pravastatin most markedly decreased the TC levels, but still there was no correlation between ΔTC ($r = 0.170$, data not shown) and ΔsICAM-1 and between ΔLDL-C ($r = 0.090$, data not shown) and ΔsICAM-1 in Group P. Thus, we speculate that pravastatin-related decreases in TC may not directly affect sICAM-1 levels, but rather pravastatin may act directly on endothelial cells to decrease ICAM-1. Previously, the West of Scotland Coronary Prevention Study [15] examined inhibition of the initial onset of coronary disease in male patients with moderate hypercholesterolemia, and reported that not only a cholesterol reduction-related mechanism, but also other mechanisms were involved in the inhibition of cardiovascular events by pravastatin, since pravastatin inhibited cardiovascular events more markedly than expected, based on the rate of cholesterol reduction. Stabilization of arteriosclerotic lesions [5], improvement in thrombocytic thrombus enhancement [10], inhibition of lipoprotein oxidation [14], and decreases in fibrinogen levels [18] have been reported as mechanisms possibly involved in the inhibition of clinical events. Furthermore, Egashira *et al.* [4] administered pravastatin to hyperlipidemic patients with coronary heart disease, and found that decreases in LDL-C inhibited acetylcholine-related coronary contractile reactions, thus increasing coronary blood flow. Therefore, improvement of vascular endothelial cell dysfunction could be involved in event inhibition [4]. Our results suggest that pravastatin inhibits cerebral infarction through plural mechanisms including decrease of TC and inhibition of cellular adhesion molecules.

ACKNOWLEDGEMENTS

The authors thank Dr. Miguel A. Suarez, Montefiore Medical Center, Bronx, NY, for reviewing the manuscript and for valuable suggestions.

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