SHORT-TERM EFFECT OF ATORVASTATIN ON THE SOLUBLE P-SELECTİN LEVEL IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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Running Title: Effect of Atorvastatin on P-Selectin

We aimed to determine the short-term effect of atorvastatin on the soluble P-selectin level in patients with hypercholesterolemia. Twenty-three patients (mean age: 60.7±11.0 yrs,19 male/4 female) with hypercholesterolemia requiring drug therapy were admitted into the study. The soluble P-selectin levels were measured using the enzyme immonoassay method. The means for plasma lipids as well as the soluble P-selectin levels before and after a 8-week treatment period were compared to each other. The mean atorvastatin dose was 24.8±13.1 mg/day (ranging from 10 to 40 mg). We found statistical differences for total cholesterol, LDL cholesterol and triglyceride but not for HDL cholesterol. While the soluble P-selectin at baseline was 1.6±0.5 ng/ml, it was 1.2±0.2 ng/ml after treatment. The difference was statistically significant (p<0.05). The change in the plasma P-selectin levels wasn't correlated with the reductions in plasma lipids. In conclusion, atorvastatin reduced the soluble P-selectin levels after a 8-week treatment period in patients with hypercholesterolemia. This reduction wasn't correlated with the changes in plasma lipids. This may be one mechanism of the anti-inflammatory effects of atorvastatin.

Key Words: Atorvastatin, P-Selectin, Hypercholesterolemia

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Hypercholesterolemia is one of the major cardiovascular risk factors. Hypercholesterolemia causes endothelial dysfunction which may result in atherosclerosis. An inflammatory reaction is a major event in the process starting with endothelial dysfunction and extending to atherosclerosis. Hypercholesterolemia has been shown to make the endothelium more suspectible to inflammation (1). In this inflammatory response, the role of adhesion molecules has been well-known. Some studies have shown that adhesion molecules are elevated in hypercholesterolemia (2-7).

The selectins, a great family of adhesion molecules, play a crucial role in the inflammatory reaction. Three types of selectins have been defined: E selectin, secreted only by the affected endothelial cells and representing endothelial dysfunction; P-selectin, secreted by the endothelial cells as well as platelets and representing endothelial dysfunction and platelet activation; and L selectin, expressed by all granulocytes, monocytes as well as most lymphocytes and standing for leucocyte activation (8). P-selectin has an important role for efficient interaction with monocytes and endothelial cells and for the activation of monocytes into macrophages (9). Because of these effects of the selectins, reducing selectin level with some specific medications such as selectin inhibitors has recently become one of the treatment goals, though this idea is still under investigation (10).

Lipid lowering therapy with statins has been thoroughly shown to regress the pathologic events triggered by hypercholesterolemia, thereby reducing cardivascular mortality and morbidity (11-13). Statins have many beneficial effects beyond lipid lowering. Statins have been determined to modify endothelial and vasomotor functions, inflammatory responses and atherosclerotic plaques. Their antiinflammatory effects may be attributed to preventing the activation of monocytes into macrophages and inhibiting the production of cytokines, C-reactive protein, and cellular adhesion molecules (14-16). In the literature, there have been a number of studies investigating the effects of several statins on various adhesion molecules (17-20). However, the number of the studies dealing with the short-term effect of atorvastatin, one of the potent statins, on the soluble P-selectin level have been limited (21-23). It is obvious that investigating this subject will mean trying to figure out the mechanism of its antiinflammatory and antiplatelet effects.

We aimed to determine the short-term effect of atorvastatin on the soluble P-selectin level in patients with hypercholesterolemia.

METHODS

Twenty-three patients with hypercholesterolemia requiring drug therapy according to the ATP III guidelines were admitted into the study. Patients with acute coronary syndrome, renal failure, hepatic dysfunction, any systemic disease and any contraindication to atorvastatin were excluded from the study. After the patient data including age, smoking status, coexisting diseases such as hypertension, diabetes and established coronary artery disease were gathered, their drug treatments was recorded, atorvostatin was prescribed at a dose indivudually determined. During the study, other medications remained unchanged. Patients were examined at baseline and at the end of a 8-week treatment period. Their blood samples were taken in the morning after overnight fasting. Serums were kept frozen at –70 C until analysed.

Plasma lipids were measured using conventional methods, and the soluble P-selectin levels were obtained with the enzyme immonoassay method using human sP-selectin ELISA kit (BioSource Europe, Belgium) following the instructions of the manufacturer.

The means for plasma lipids as well as the soluble P-selectin levels before and after a 8-week treatment period were compared to each other. The means were expressed as mean ± SD. The Wilcoxon test was used for comparisons. The correlation was saught using the Pearson correlation co-efficient. A p value under 0.05 was considered significant. The comparisons were done using the SPSS 10.0 for Windows program.

The study was approved by The Local Ethics Committee. All patients gave written consent.

RESULTS

The mean age was 60.7 ± 11.0 yrs. The characteristics of the study population were summarized in Table 1. The mean atorvastatin dose was 24.8 ± 13.1 mg/day (ranging from 10 to 40 mg). Table 2 showed plasma lipids at baseline and after the 8-week treatment. We found statistical differences for total cholesterol, LDL cholesterol and triglyceride but not for HDL cholesterol.

Table 1. The Characteristics Of The Study Population

Study population (n=23)	Number of patient(%)
Male/female	19 (82.6)/ 4 (17.4)
Smokers	5 (21.7)
Existing diseases	
Coronary artery disease	14 (60.8)
Hypertension	14 (60.8)
Diabetes	3 (13.0)
Medications	
Acetylsalicylic acid	23 (100)
ACE inhibitors/ATII antagonist	13 (56.5)
Ca-channel bloker	4 (17.4)
Beta Bloker	15 (65.2)
Long-acting nitrate	13 (56.5)

ACE: Angiotensin converting enzyme, AT II: Angiotensin II

Table 2. Plasma Lipids At Baseline And After The 8-Week Treatment

Parameters (mg/dl)	Baseline (mean±SD)	8 weeks (mean±SD)	p
Total cholesterol	250.4 ± 49.1	179.4 ± 42.6	<0.05
LDL-cholesterol	174.4 ± 35.5	112.6 ± 41.1	<0.05
HDL-cholesterol	43.7 ± 7.6	43.9 ± 7.0	>0.05
Tryglyceride	133.1 ± 60.1	103.7 ± 45.9	<0.05

SD: Standard deviation

While the soluble P-selectin at baseline was 1.6 ± 0.5 ng/ml, it was 1.2 ± 0.2 ng/ml after treatment. The difference was statistically significant ($p<0.05$). The means and comparisons were shown as a box-plot graphic in figure 1. The change in the plasma P-selectin levels wasn't correlated with the reductions in plasma lipids.

DISCUSSION

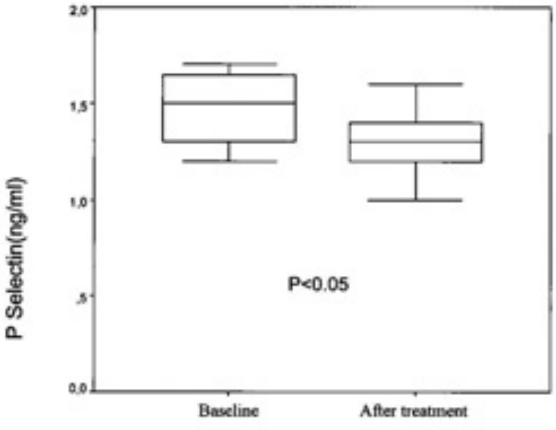

Figure 1. Soluble P-Selectin at Baseline and After Treatment as A Box-Plot Graphic

Our results revealed that atorvastatin lowered plasma lipids including total cholesterol, LDL-cholesterol and tryglyceride as well as the soluble P-selectin level in patients with hypercholesterolemia after a 8-week treatment period. P-selectin is stored in --granules of platelets and in Weibel-Palade bodies of endothelial cells. Activated platelets express P-selectin which binds P-selectin glycoprotein ligand-1 on leucocytes and monocytes. This reaction is reponsible for the recruitment of inflammatory leucocytes to thrombi (8). It was shown that plasma P-selectin level was elevated during acute coronary syndromes (24,25). Also, P-selectin is required for the activation of monocytes into macrophages. It is obvious that increased P-selectin represents platelet activation and endothelial dysfunction. Therefore, P-selectin plays a crucial role in atherosclerosis pathogenesis (9). Moreover, it was indicated that adhesion molecules are increased in hypercholesterolemia in some studies. Hypercholesterolemia enhances the expression of endothelial cell adhesion molecules and renders the endothelium more suspectible to inflammation (2-7). Davi et al. showed that hypercholesterolemia was associated with elevated plasma P-

selectin. They claimed that an altered oxidative process and persistent platelet activation may contribute to increased soluble P-selectin levels (7). Furthermore, increased P-selectin may be proposed as a vascular risk factor. A study by Ridler et al investigating the value of soluble P-selectin in determining vascular risk indicated that healty women with elevated P-selectin levels had more risk for future vascular events (26). It is apparent that reducing soluble P-selectin levels would mean preventing the inflammatory response of vascular endothelium, modifying platelet function and reducing vascular risk. With our results, it may be suggested that atorvastatin has these effects by reducing the soluble P-selectin levels in patients with hypercholesterolemia. In our study, we focused on the effect of atorvastatin treatment on the soluble P-selectin level in hypercholesterolemia regardless of whether the subjects had elevated P-selectin levels or not. In fact, we didn't test whether the subjects had elevated P-selectin levels in hypercholesterolemia. Also, we didn't group the patients as to their association with any disease such as hypertension, coronary artery disease or diabetes, their levels of P-selectin, that is, elevated or not, or the dose of atorvastatin they receieved. Indeed, our population was too small to group the patients in terms of these types of features. However, hypercholesterolemia is frequently associated with coronary heart disease, hypertension, diabetes etc. Hence, beneficial effects of statins are expected for all patients. For that reason, we didn't group the patients according to their some features mentioned above.

It has been shown that statins have anti-inflammatory properties beyond lipid lowering in the large trials as well as in experimental animals and cell culture systems (11-16). LDL cholesterol represents potent pro-inflammatory stimuli, and it is clear that lowering LDL cholesterol normalises systemic inflammatory markers. Also, an increase of HDL cholesterol with statin treatments is a contributing factor in reducing inflammatory markers (14). However, in the studies, a correlation between LDL cholesterol changes and C reactive protein wasn't found (27). The role of adhesion molecules in inflammation has been well-known (8). The effects of statins on adhesion molecules are still being investigated. Although statins have been shown to reduce some selectins, there have been some reports indicating that some statins have a different effect on other adhesion molecules, such as intercellular adhesion molecule-1(ICAM-1), vascular-cellular adhesion molecule-1(VCAM-1) and E-selectin (28,29). Empen et al reported that a 4-week atorvastatin treatment didn't affect on the level of adhesion molecules including E-selectin, ICAM-1 and VCAM-1 in patients with hypertryglyceridemia (30). In that study, the study population was different from our population, and P-selectin wasn't determined. In a recent study, Nawawi et al investigated the effect of atorvastatin on adhesion molecules including E-selectin, ICAM-1 and VCAM-1 in patient groups with familial or nonfamilial hypercholesterolemia, and they reported that there were reductions in serum ICAM-1 levels at 2 weeks, 3 months and 9 months for both groups. In that study, there was reduction in serum VCAM-1 levels at 3 months but not at 9 months and only in the nonfamilial hypercholesterolemia group. It was also reported that a reduction in the E-selectin levels was observed at 9 months in both groups (31). An other study by Nawawi et al indicated that low dose atorvastatin treatment in patients with nonfamilial hypercholesterolemia reduced adhesion molecules at 2 weeks and 3 months for ICAM-1, VCAM-1 but not for E-selectin (32). P-selectin levels were not determined in these studies by Nawawi et al. Moreover, Jilma et al reported that the levels of ICAM-1,VCAM-1, E-selectin did not decrease after 3 months of statin therapy with

atorvastatin, simvastatin, pravastatin in moderate hypercholesterolemia (29). However, Purcetti et al determined time-dependent effects of statins including atorvastatin on platelet function in hypercholesterolemia by measuring surface P-selectin, and they claimed that atorvastatin reduced platelet activity after 1,2,3 and 4 weeks of treatment (22). Also, Seljeflot et al investigated the effect of atorvastatin and simvastatin on adhesion molecules inculding the ICAM-1, VCAM-1, E-selectin and P-selectin levels after 1 year treatment in patients with coronary heart disease, and they reported that atorvastatin lowered all cellular adhesion molecules, reaching statistical significance for ICAM-1 and P-selectin. In that study, statistical significance was observed for E selectin and P-selectin, whereas a statistically significant increase in VCAM-1 was seen in the simvastatin group (21). When combining their results with ours, it might be expressed that the reducing effect of atorvastatin on P-selectin levels may be observed at early stage of treatment (8 weeks), though our population was slighty different from those in the study by Seljeflot et al. In an other study by Atalar et al, it was reported that atorvastatin reduced the soluble L-selectin level in hyperlipidemic patients with coronary heart disease at 12 weeks (33). All findings indicate that atorvastatin has antiinflammatory and antiplatelet effects, though with varying results. The existence of different results may imply that the interaction of adhesion molecules with statins and the mechanisms of their antiinflamatory properties are more complex than appears, and these subjects need to be dealt with in more further investigations. Also, not finding a correlation between lipid changes and the P-selectin reduction with treatment may be said to confirm to this idea. With this point of view, we think that our study can be a part of the collected data regarding the effects of atorvastatin.

In the literature, there have been some studies regarding the effects of other statins including simvastatin, fluvastatin, pravastatin on soluble P-selectin levels. It has been reported that these statins reduce soluble P-selectin levels at early stage of treatment (4-12 weeks) in hypercholesterolemia (17-20). With these findings, it is likely to be suggested that the lowering effect on the soluble P-selectin level may be a group effect of statins.

In conclusion, atorvastatin reduced the soluble P-selectin levels after a 8-week treatment period in patients with hypercholesterolemia. This reduction wasn't correlated with the changes in plasma lipids. This may be one mechanism of the antiinflammatory effects of atorvastatin.

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