Abstract: It has been hypothesized that treating hypercholesterolemic patients with statins will lead not only to a reduction in cholesterol, but also to inhibited synthesis of other compounds that derive from the synthetic pathway of cholesterol. One important by-product is ubiquinone (CQ), which has a pivotal role in mitochondrial electron transport and antioxidant activity. We therefore investigated the effect of 2 months of simvastatin treatment (20 mg/day) on blood ATP concentration, plasma total antioxidant capacity and ubiquinone levels in 17 hypercholesterolemic patients (age range: 40 to 65). To evaluate the possible muscle-related side effects, plasma CK activity and myoglobin concentrations were compared before and after therapy. We observed decreased plasma ubiquinone levels and total antioxidant capacity after two months of therapy. CK activity and myoglobin concentrations were increased in the treated group. There was not statistically significant difference for whole blood ATP levels. It may be concluded that simvastatin lowers plasma ubiquinone concentrations. The possible adverse effect of simvastatin on ubiquinone metabolism may be clinically important and requires further study.

Key Words: Ubiquinone, HMG CoA reductase inhibitors, ATP, antioxidant

Introduction

3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, or statins, have been widely used to lower hypercholesterolemia. They inhibit the conversion of HMG-CoA to mevalonic acid, the second step in cholesterol biosynthesis, and lower plasma cholesterol (1,2). However, a number of studies have reported possible psychiatric adverse effects and cancer associated with cholesterol lowering (3,4). Other isoprene compounds such as ubiquinone synthesized in the same pathway may also be affected by statins (5).

An increase in psychiatric adverse effects and cancer has been reported at low levels of cholesterol (4,5). Being a member of the electron transport chain, ubiquinone plays an important role in ATP synthesis, and, in its reduced form, it protects membranes against free radical damage with its antioxidant features (6,7). Therefore, its reduced levels may be clinically relevant. Some authors have suggested that, due to the role of ubiquinone in the electron transport chain, treatment with statins would also lead to a reduction in ATP production (8).

Although the major site of action of HMG CoA reductase inhibitors in lowering serum cholesterol is the hepatocyte, the lipophilic nature of simvastatin results in decreased selectivity and uptake of the drug by various cell types (9). Clinical evidence indicates a potential for skeletal muscle toxicity including elevated creatine kinase levels, myositis, and even rhabdomyolysis after therapy with HMG CoA reductase inhibitors. Myopathy has been found, especially during combination therapy with other drugs such as gemfibrozil, cyclosporin and erythromycin (10).

The aim of this study was to determine the effects of treatment with simvastatin, a potent HMG CoA reductase inhibitor, on plasma ubiquinone, whole blood adenosine triphosphate (ATP) levels, total antioxidant capacity and muscle related markers at usual doses.

Materials and Methods

Subjects

Seventeen hypercholesterolemic men, aged 40 to 60 years, for whom simvastatin therapy was started by the Dokuz Eylül University Medical Faculty Cardiology Outpatient Clinic, volunteered for this study. Their plasma total cholesterol concentrations ranged between 6.06 and 10.4 mmol/L. They were otherwise healthy and had never received hypocholesterolemic or other drug therapy.
They were instructed to adhere to their normal diet and not to use vitamin supplements during the study. All hypercholesterolemic patients received 20 mg simvastatin nightly for 2 months. The blood samples were collected before and after the treatment period.

Fifteen apparently healthy men with a total plasma cholesterol level below 5.8 mmol/L were selected as the control group. They were age matched and took no medications and had no signs of disease. The study was approved by the ethical committee of the university and all the men gave their informed consent.

Laboratory methods

Fasting blood samples were drawn for lipid, lipoprotein, ubiquinone, ATP, CK, myoglobin and total antioxidant capacity determinations.

Lipid and lipoprotein analyses. The concentrations of total cholesterol, HDL cholesterol and triglycerides in plasma were determined using commercial kits manufactured by Sigma Diagnostica. LDL cholesterol was calculated using the Friedewald equation (11).

Ubiquinone. The ubiquinone assays were performed by a high-performance liquid chromatographic method as described by Grossi et al. (12), using a C-18 reversed-phase column 5 µm(125 mm x 4.6 mm I.D.) and a mobile phase of 2-propanol-methanol (1:4) with ultraviolet detection at 275 nm.

ATP. Whole blood ATP levels (13) were measured immediately after blood sampling. ATP analysis was performed after deproteinization with trichloroacetic acid with an enzymatic, colorimetric ATP assay kit from Sigma Diagnostica.

Total antioxidant capacity (TAC). Plasma TAC was measured using the ABTS (Azino-diethyl-benzthiazoline sulfate) method (14) with a total antioxidant status kit from Randox Laboratories Ltd.

CK. CK activity was measured with a quantitative, kinetic kit manufactured by Sigma Diagnostica on a Hitachi 747-200 autoanalyzer.

Myoglobin. Myoglobin analysis was performed with an immunochemical method using myoglobin kits manufactured by Boehringer Mannheim Diagnostica (Mannheim, Germany) on an Elecsys-2010 autoanalyzer.

Statistical analysis

The SPSS program was used for statistical analyses. The values were expressed as mean (SD). The significance of differences between groups was determined by Wilcoxon paired test.

Results

After 2 months of treatment with simvastatin, plasma total and LDL cholesterol levels decreased from pretreatment levels on average by 29% and 38% respectively (p < 0.001). In addition, a decrease in plasma triglycerides (33%; p < 0.001) and a slight increase in plasma HDL cholesterol (+8%; p = 0.18) were observed (Table 1).

In the simvastatin group, a decrease of 30% (p < 0.001) was detected in plasma total ubiquinone after 2 months of treatment, but these concentrations (0.90 mg/L) were still higher than those in the control group (0.62 mg/L; p < 0.01) (Table 1). The close relationship between ubiquinone(CQ) and cholesterol levels was reflected by the constant CQ:total cholesterol and CQ:LDL cholesterol ratios before and after therapy (Table 1).

There were no significant differences in blood ATP levels before and after therapy while total antioxidant capacity decreased after therapy (p < 0.01) (Table 2). Significant increases were noted for plasma CK activity (p < 0.001) and myoglobin concentrations (p < 0.001) after therapy (Table 3).

Discussion

The results of our study showed that plasma ubiquinone levels were significantly lower after the hypercholesterolemic patients were treated with simvastatin. Our results agree with previous studies suggesting that serum ubiquinone levels are considerably higher in patients with untreated hypercholesterolemia than in normocholesterolaemic individuals (15,16). In some previous studies, it has been reported that although statin treatment lowers serum ubiquinone values, the reduced values still remain at the same or even higher levels than those of healthy normolipemic subjects (15,16). The results of the present study further support this concept. The decrease in ubiquinone levels was parallel to the decrease in total cholesterol and LDL cholesterol as also reported in other studies (15,16). The close relationship between plasma ubiquinone and cholesterol levels was reflected by the invariable ubiquinone-cholesterol relationship regardless whether
The patients were receiving statin or not. Furthermore, these ratios in the normal control subjects were comparable to those observed in the statin-treated patients. The decrease in ubiquinone levels could either be due to the inhibition of its biosynthesis or to the reduced amounts of low density lipoproteins that transport it, or both factors might have contributed to it.

Mainly based on findings of lowered serum ubiquinone levels during statin treatment, it has been hypothesized that mitochondrial generation of high-energy phosphates could be adversely affected in statin-treated patients (17,18). An experimental study in simvastatin-treated rats reported a decrease in blood ATP levels but no impairment in muscle high-energy phosphate metabolism (19). Similarly, Laaksonen et al. reported that skeletal muscle high-energy phosphates did not change in hypercholesterolemic patients receiving 6 months of simvastatin treatment (20). The results
showed that whole blood ATP concentrations were not affected following 2 months (20/mg/day) of simvastatin therapy.

However, since ubiquinone is present in mitochondria in molar amounts greatly exceeding those of other respiratory chain carriers (21), the rate of ATP production in long-term simvastatin-treated patients are not clear yet and needs further investigation.

Muscle necrosis and high serum CK activities were reported in simvastatin treated rabbits (22). Our results also indicate that plasma CK and myoglobin concentrations were increased after simvastatin therapy. Several explanations for the increase in CK have been proposed. It has been stated that ubiquinone deficiency could result in a mitochondrial myopathy with reduced mitochondrial ATP production and mitochondrial dysfunction. Chariot has reported that a 63-year-old female in whom simvastatin has induced rhabdomyolysis and mitochondrial myopathy and whose muscle ubiquinone levels were below normal and symptoms improved with ubiquinone replacement (23).

Another possible mechanism that can explain the myopathy is that simvastatin interferes with muscle cholesterol synthesis, eventually altering the lipid composition and microviscosity of the membranes of muscle cells. Simvastatin, like other lipophilic HMG CoA reductase inhibitors, is not liver selective and enters cells of various tissues like muscle tissues (24). Clinical experience has shown that the concomitant use of other drugs (i.e. macrolide antibiotics and immunosuppressants) with simvastatin increase the risk of myopathy (25,26). The findings of our study indicate that the use of simvastatin alone and at usual doses leads to increased CK and myoglobin levels. For this reason, it may be concluded that patients treated with simvastatin should be monitored for CK and myoglobin and if a significant elevation is detected in patients using simvastatin, the drug may be changed since highly tissue selective drugs such as pravastatin are reported not to induce muscle toxicity (27).

In addition to its well-established function as a component of the mitochondrial respiratory chain, ubiquinone has in recent years acquired increasing attention with regard to its function in reduced form (ubiquinol) as an antioxidant. Ubiquinol is the only known lipid-soluble antioxidant that animal cells can synthesize de novo. Its high degree of hydrophobicity and its widespread occurrence in biological membranes and in low-density lipoproteins suggest an important role of ubiquinol in cellular defense against oxidative damage (28). Ubiquinol is suggested to have an antioxidant function both directly by radical scavenging and also indirectly by acting as an electron donor for a-tocopheroyl radicals (a-TO.), thus providing endogenous tocopherol (vitamin E) regeneration and enhancing overall antioxidant activity in membranes (29,30). In our study, we observed a significant decrease in TAC following simvastatin therapy. This could be explained by the decrease in ubiquinone related to the possible mechanism discussed above. To our knowledge, this is the first study of TAC in simvastatin-treated patients.

A number of studies have demonstrated a positive correlation between low serum cholesterol levels and noncardiovascular deaths due to cancer, accidents, and depression (31,32). However, this association has not been explained yet. In recent larger clinical trials, these early results were not confirmed (33,34). The presence of such a connection remains controversial.

For patients with known coronary heart disease, the recent Scandinavian Simvastatin Survival Study suggests that the benefits of cholesterol lowering exceed the risks, at least in men and in the short term (35).

However, decreased ubiquinone levels have been reported in several diseases including neurodegenerative diseases and cancer (36-39). It is also not clear yet whether and to what extent these diseases are related to an alteration of the bioenergetic capacity and/or of the antioxidant status of the tissues concerned. Thus, it may be questioned whether the decrease is responsible for the adverse effects of lowered cholesterol levels in simvastatin-treated patients.

In conclusion, our results show that simvastatin does lower plasma ubiquinone levels, even in short-term therapy. Because of its important role for human organism we think that the supplementation of simvastatin-treated patients with ubiquinone would be beneficial.

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