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Oxidation Injury in Patients Receiving HMG-CoA Reductase Inhibitors

Occurrence in Patients Without Enzyme Elevation or Myopathy

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Abstract

Background: Myopathy in its severe forms including rhabdomyolysis is a very rare adverse effect occurring during monotherapy with the HMG-CoA reductase inhibitors ('statins') and is associated with pronounced signs of oxidation injury. This has been found at a local (muscle) as well as at a systemic level (blood). Several lines of evidence indicate that even mild forms of myopathy during statin treatment may be associated with *in vivo* oxidation injury. In contrast, statin therapy has been shown to be associated with a decrease in oxidation injury.
Objective: The aim of this study was to investigate whether patients with heterozygous familial hypercholesterolaemia who did not exhibit any symptoms or abnormalities in safety parameters during 6 months of treatment with various statins (atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin) did exhibit a change in oxidation injury as assessed by the isoprostane levels.
Methods: Blood (plasma and serum) as well as urine was tested before and 1, 3 and 6 months after starting statin therapy.
Results: Out of 111 treated patients (63 males, 48 females; aged 19 to 58 years) who did not experience any adverse effects during statin treatment, 11 (seven males, four females; aged 24 to 51 years) showed a pronounced increase in 8-epi-prostaglandin (PG) $F_{2\alpha}$ in all the compartments examined. In the remaining 100 patients (56 males, 44 females; aged 19 to 58 years) there was either no change in or even an apparent decrease in 8-epi-$PGF_{2\alpha}$. This increase was monitored with all the statins administered. If elevated, the increase in 8-epi-$PGF_{2\alpha}$ remained without change throughout the entire follow-up period. No sex difference or differential response between smokers and nonsmokers was observed.
Discussion: These findings indicate that in the absence of other clinically observable adverse effects, in some of the patients, for an as yet unknown reason, statin therapy may be associated with increased oxidation injury. The fact that changing to another statin is apparently not necessarily associated with an identical response raises the question of a specific predisposition for certain compounds in a given patient. These data add a further piece of evidence that mild adverse effects of statins that are difficult to assess might be much more prevalent than widely considered. The clinical relevance and consequence of these findings still remains to be assessed.

Background

One of the major adverse effects occurring during long-term treatment with HMG-CoA reductase inhibitors or 'statins' in patients with familial hypercholesterolaemia (FH) is myopathy.[1] Recently, it has been found that clinical symptoms may occur even in the absence of creatine kinase (CK) elevation.[2] Although the underlying mechanism is still unclear, several pieces of evidence indicate that oxidation injury may be involved to a certain extent. Statins may decrease, in an unknown way, ubiquinone levels[3] resulting in a decreased oxidation injury defence of lipoproteins.[4] Ubiquinone[5] and mevalonate[6] administration has been shown to reverse clinical symptoms. Recently, we discovered that in a certain number of patients receiving treatment with statins, 8-epi-prostaglandin (PG) $F_{2\alpha}$ levels are increased in serum, urine and plasma.[7] A case report indicated that tocopherol (vitamin E) treatment not only reversed the symptoms of myopathy but also normalised plasma levels of 8-epi-$PGF_{2\alpha}$.[8] Rhabdomyolysis has been shown to be associated with an increased isoprostane (IP)-formation,[9] while in a certain subgroup of patients with myopathy (with or without CK-elevation) 8-epi-$PGF_{2\alpha}$ is increased.[10,11] Normally, statin therapy reduces 8-epi-$PGF_{2\alpha}$ [12] in patients with hypercholesterolaemia.

Objective

The objective of this study was to investigate whether long-term statin treatment in patients with heterozygous FH without any symptoms and abnormalities in safety parameters experienced changes in 8-epi-$PGF_{2\alpha}$ levels.

Materials and Methods

Patients with heterozygous FH in whom statin therapy was initiated were involved in this study. All statins that were available in Austria at the time of the study, i.e. atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, were prescribed (cerivastatin was not available). Data on 111 consecutive patients without subjective symptoms (muscle pain) and with normal safety parameters at all of the time intervals were included in the study. Characteristics of these patients are presented in table I. Patients not fitting the inclusion criteria were excluded. Except for smoking and FH, no other risk factors for the developement of atherosclerosis were present. Concomitant drugs and eating behaviour (especially concerning the use of herbal medicines, herbals teas etc.) remained unchanged throughout the monitoring period. Patients did not take vitamins or drugs known to interfere with antioxidant status. Lipids (total cholesterol, triglycerides) and lipoproteins (high-density lipoprotein, low-density lipoprotein and very-low-density lipoprotein) as well as 8-epi-$PGF_{2\alpha}$ in serum, plasma and urine were determined before initiating drug treatment as well as after 1, 3 and 6 months of treatment with the respective statin. A threshold increase of <20% in 8-epi-$PGF_{2\alpha}$ was chosen and considered as abnormal response because long-term follow-up showed that in >98% of people a <15% change in 8-epi-$PGF_{2\alpha}$ occured. Patients were given a questionnaire on statin-induced muscle pain asking questions about type (ache, cramp), appearance at rest or exercise, site, onset, duration, time of day and others.

Blood was drawn in the morning after an overnight fasting period of at least 12 hours and a 30 minute rest. Lipids, lipoproteins, safety parameters (AST, ALT, CK, γ-glutamyl transferase and lactate dehydrogenase), creatinine and 8-epi-$PGF_{2\alpha}$ were measured. A 24-hour urinary sample was collected at the same time and, after assessing total urinary volume, an aliquot was stored at –70°C for determination of 8-epi-$PGF_{2\alpha}$. Urinary creatinine and clearance were determined in parallel.

Serum 8-Epi-Prostaglandin (PG) $F_{2\alpha}$

Blood was drawn into glass vials. Vials were placed immediately into a water bath at 37°C for exactly 60 minutes. Serum was then removed after

Table I. Patient characteristics

Groups	All study participants			Subgroup showing an increase in 8-epi-$PGF_{2\alpha}$		
	total (n = 100)	men (n = 56)	women (n = 44)	total (n = 11)	men (n = 7)	women (n = 4)
Age (years)	19-58	19-54	22-58	24-51	24-51	24-50
SM/NS	29/61	20/36	9/35	4/7	2/5	2/2
FH (years since diagnosis)	0-12	0-10	1-11	0-12	1-8	0-12
ATH +/–	24/76	15/41	9/35	3/8	2/5	1/3
Other drugs +/–	37/63	22/34	15/29	3/8	1/5	2/3

ATH = clinical manifestation of atherosclerosis; **8-epi-$PGF_{2\alpha}$** = 8-epi-prostaglandin $F_{2\alpha}$; **FH** = familial hypercholesterolaemia; **NS** = nonsmokers; **SM** = smokers.

a centrifugation step (4°C, 1000 × g, 10 minutes) and stored until determination (no longer than 2 weeks at <–70°C) as described.[12] 8-Epi-$PGF_{2\alpha}$ was determined after extraction and purification by chromatography. *In vitro* artefactual formation of 8-epi-$PGF_{2\alpha}$ (which eventually could be easily generated by *in vitro* auto-oxidation of arachidonic- or other fatty acids) was excluded by comparison with immediate measurements showing no difference in the respective eicosanoid. To determine inter-assay variability, the respective sample was determined several times in several assays. The intra-assay variability was determined assaying the same sample several times during the same assay procedure. The inter-assay variability amounted 3.8 ± 1.2%, the intra-assay variability was 1.9 ± 0.7%. The normal value for serum 8-epi-$PGF_{2\alpha}$ is 150 to 250 ng/L (n = 17).

Plasma 8-Epi-$PGF_{2\alpha}$

Blood samples were anticoagulated with 2% ethylene diamine tetra-acetic acid and 1 mg/ml (final blood volume) aspirin (acetylsalicylic acid). Immediate centrifugation at 4°C to obtain plasma was done at 1000 × g for 10 minutes. Plasma was removed and stored at < –70°C for not longer than 2 weeks until determination. Probes were sampled and processed using plastic material only. The inter-assay variability was 5.5 ± 1.7%, the intra-assay variability 2.5 ± 0.7%. The normal value for plasma 8-epi-$PGF_{2\alpha}$ is <20 ng/L (n = 11).

Urinary 8-Epi-$PGF_{2\alpha}$

Urine was collected over a period of 24 hours. 10ml aliquots were adjusted to pH 4.0 with formic acid and taken for extraction. The eluate was subjected to silicic acid chromatography and further eluted. This final eluate was dried, recovered in buffer and assayed after dilution. Cross reactivity of the antibody with prostaglandins was <2%. Values are given in picograms of 8-epi-$PGF_{2\alpha}$ per milligram of creatinine. The inter-assay variability was 6.4 ± 2.3%, the intra-assay variability 2.7 ± 0.8%. The normal value for urinary 8-epi-$PGF_{2\alpha}$ is 150 to 250 pg/mg creatinine (n = 14).

Routine Safety Parameters

Routine safety parameters (AST, ALT, γ-glutamyl transferase, lactate dehydrogenase, CK) were determined by routine laboratory methods.

Statistical Analysis

Values are presented as mean ± SD; calculation for significance was done using analysis of variance. A p-value of <0.01 was considered as significant.

Results

Out of 111 patients treated with various statins (36 with simvastatin, 32 with atorvastatin, 18 with pravastatin, eight with lovastatin, and six with fluvastatin), 11 (see table II for characteristics) exhibited an increase in 8-epi-$PGF_{2\alpha}$ at all the time

Table II. Characteristics of patients (n = 11) showing an increase in 8-epi-prostaglandin $F_{2\alpha}$

Patient no.	Sex	Age (years)	Height (cm)	Bodyweight (kg)	FH (years since diagnosis)	ATH (+/–)	SM (+/–)	Other drugs (+/–)	Statin
1	m	41	181	77	8	–	–	–	Lovastatin
2	f	46	169	60	9	–	–	+	Simvastatin
3	m	24	187	80	4	–	+	–	Atorvastatin
4	m	37	163	55	7	–	–	–	Fluvastatin
5	m	36	177	77	1	–	–	–	Simvastatin
6	f	43	158	53	10	–	–	–	Pravastatin
7	f	24	162	60	0	–	+	–	Atorvastatin
8	m	51	174	76	7	+	+	+	Atorvastatin
9	m	42	179	73	5	+	–	–	Simvastatin
10	f	50	159	54	12	+	+	+	Atorvastatin
11	m	33	191	87	3	–	–	–	Pravastatin[a]

a Always used at the lowest available dose.

ATH = clinical manifestation of atherosclerosis; **f** = female; **FH** = familial hypercholesterolaemia; **m** = male; **SM** = smokers

points measured versus the respective pretreatment value (see table III). In none of the patients was an elevated value only at one of the time points. The increase occured within the first month of therapy and there were no further increases after this time. Of the patients, 8.33% taking simvastatin, 12.50% taking atorvastatin, 11.11% taking pravastatin, 12.50% taking lovastatin and 16.67% taking fluvastatin showed an increase in 8-epi-$PGF_{2\alpha}$. The size of the increase in 8-epi-$PGF_{2\alpha}$ was comparable in females (9.1%, 4 out of 44) as in males (12.5%, 7 out of 56). The pretreatment values were higher in smokers than in nonsmokers, the change on statin therapy, however, was almost identical (table IV). In all the patients where an increase was observed this increase was seen in plasma, serum and urine as well.

Discussion

There is some evidence that statin-induced myopathy may at least in part be due to oxidation in-

Table III. 8-Epi-prostaglandin $F_{2\alpha}$ values in the patients with abnormal response values

Patient no.	Plasma (pg/ml)				Serum (pg/ml)				Urine (pg/mg creatinine)			
	p	1mo	3mo	6mo	p	1mo	3mo	6mo	p	1mo	3mo	6mo
1	27	36	42	39	262	348	380	397	353	497	521	530
2	24	40	43	42	256	371	395	368	331	477	502	486
3	38	75	73	80	457	694	731	712	497	726	851	783
4	28	40	37	37	271	365	370	364	362	469	471	486
5	24	36	43	47	265	362	384	371	316	462	484	465
6	26	46	49	45	290	381	362	387	327	498	483	492
7	35	53	56	57	428	596	561	612	473	514	564	570
8	42	63	61	66	477	612	641	670	483	609	625	621
9	29	37	56	54	284	375	542	508	342	486	554	570
10	39	44	40	39	329	446	425	429	409	502	461	462
11	29	51	47	54	308	575	544	569	344	542	536	579

p = pretreatment value.

Table IV. 8-Epi-prostaglandin (PG) $F_{2\alpha}$ in smokers (SM) versus nonsmokers (NS) not showing an increase in isoprostane levels (± standard deviation)

Smoking status	Medium	Pretreatment value	Value at 1 month[a]	Value at 3 months	Value at 6 months
NS (n = 61)	Plasma	28.3 ± 7.3	24.1 ± 5.0	23.7 ± 4.6	23.8 ± 4.7
	Serum	286.7 ± 37.2	262.4 ± 29.8	257.8 ± 31.4	259.4 ± 27.9
	Urine	351.4 ± 42.5	294.3 ± 36.2[b]	288.5 ± 34.6	290.7 ± 35.2
SM (n = 39)	Plasma	36.4 ± 8.9	30.7 ± 7.2	30.4 ± 6.8	29.6 ± 6.9
	Serum	457.3 ± 48.2[c]	335.0 ± 39.2[b,c]	341.7 ± 40.6[b,c]	334.2 ± 39.1[b,c]
	Urine	486.5 ± 56.2[c]	408.2 ± 57.9[b,c]	411.9 ± 60.2[b]	400.9 ± 51.3[b,c]

a After 1 month there was already a (partly significant) decline in 8-epi-$PGF_{2\alpha}$ level without further change later on in the patients showing the typical pattern.

b $p < 0.01$ (versus pretreatment value).

c $p < 0.01$ (versus nonsmokers).

jury. Some of the adverse events of statins may be due to the inhibition of ubiquinone biosynthesis, an essential coenzyme for many intra- and extracellular functions.[13] It has been shown that levels of ubiquinol-10, the reduced form of ubiquinone, are lower in patients with hyperlipidaemia. Its determination was used to assess oxidative stress.[14] Supplementation of ubiquinone increased its level within lipoproteins resulting in resistance against oxidation injury.[15] Decreased levels of ubiquinone in plasma were reported after treatment with lovastatin,[16] simvastatin[17] and pravastatin[18] and decreased levels were also seen in platelets following treatment with simvastatin.[13]

In several case reports, supplementation with ubiquinone was reported to reverse muscle injury.[3,4] The finding of clinical improvement in a patient after adding tocopherol[8] though continuing statin therapy supports the concept of oxidation injury. Another parameter that can be used to detect *in vivo* oxidation injury is the isoprostanes.[19] The isoprostane 8-epi-$PGF_{2\alpha}$ is formed during LDL-oxidation.[20] Isoprostanes may play an important role in lesion formation and progression.[21,22] Increased 8-epi-$PGF_{2\alpha}$ in vascular tissue and particularly in foam cells has been found by several groups.[22,23] Increased isoprostane and oxidation injury may show lesion promoting effects. Under normal circumstances, lipid lowering by statins reduces 8-epi-$PGF_{2\alpha}$.[12] There is no diurnal variation in urinary levels of 8-epi-$PGF_{2\alpha}$.[24] A control group in an earlier study[12] revealed no changes. In 57 patients with hyperlipidaemia, who were not receiving therapy, there was no change in 8-epi-$PGF_{2\alpha}$ over a 6 month period (unpublished observations). Intraindividual day-to-day variations are low allowing assessment of follow-up measurements. This finding was confirmed in the 100 patients in this study who did not experience an increase in 8-epi-$PGF_{2\alpha}$ with statin therapy. In a subgroup of patients showing adverse effects with statins, an increase rather than the expected decrease was observed.[10,11] This is the first report showing that even in absence of any classical adverse effect, there may be a kind of oxidation injury in a smaller subgroup of patients.

Is this response substance specific? Changing the statin in one patient (patient 8; see table II) after 6 months of therapy from atorvastatin to pravastatin did not change 8-epi-$PGF_{2\alpha}$ levels, while a pause in therapy of 4 weeks normalised the isoprostane to pretreatment values. We do not have this information yet for any other patients. In five patients not belonging to this study group, who exhibited an increase in 8-epi-$PGF_{2\alpha}$ (plasma: 57 ± 8 ng/L; serum: 556 ± 64 ng/L; urine: 512 ± 49 pg/mg creatinine) without any clinical symptoms of myopathy, withdrawal of statin therapy resulted in a complete normalisation (plasma: 29 ± 3 ng/L; serum: 302 ± 24 ng/L; urine 339 ± 41 pg/mg creati-

nine) of the isoprostane within 14 days at the latest. Restarting statin therapy caused an increase within a few days only, reaching a maximum at about 1 week. A change to another statin in one of the five patients was not accompanied by an increase in isoprostanes indicating that particular patients might respond differently to various members of this family of drugs (unpublished observations).

Data from 8-epi-$PGF_{2\alpha}$ studies in patients showing clinical adverse effects, however, revealed, that an elevation potentially occurred without any detectable biochemical background.[11]

Our data indicate that in a subgroup of patients there is evidence of oxidation injury. Whether this subgroup develops myopathy later on or continues with subclinical myopathy is unknown.

These findings raise the question of whether an increase in 8-epi-$PGF_{2\alpha}$, even in the absence of any clinical symptom, could have consequences. Should this kind of measurement be considered as part of routine statin monitoring? And if so, which measurements? Should therapy be stopped on the basis of 8-epi-$PGF_{2\alpha}$ elevation or should therapy with tocopherol, ascorbic acid (vitamin C), beta-carotene, ubiquinone or mevalonate be started? Or is this effect comparable with the elevation of 8-epi-$PGF_{2\alpha}$ seen in cigarette smokers[7] i.e. it is clinically negligible?

The data presented here add a further piece of evidence, that mild adverse effects of statins may be more frequent than previously thought. Detailed metabolic studies are required to shed more light on to the underlying mechanisms in the future.

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