


The Carotid Intima–Media Thickness Modification Following Atorvastatin is Bound to the Modification of the Oxidative Balance

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Abstract

Background: Despite the reduction in cholesterol (CH) levels, the modification of carotid intima–media thickness (CIMT) is not evident in all the patients treated with statins. Activities other than CH reduction may determine the improvement in CIMT. **Methods:** Twenty-two patients with hypercholesterolemia (aged 45–60; males) with CIMT increase started the treatment with atorvastatin. The CIMT (via echography), CH level, and the oxidative balance (OB) were measured at baseline and after 4 weeks. The OB consisted of the determination of the plasmatic hydroperoxides (reactive oxygen metabolites [d-ROMs] test) and the antioxidant reserve (plasma antioxidants test [PAT]). The d-ROMs/CH and PAT/CH ratios allowed to measure, respectively, the oxidative index (OI) and the protective index (PI). The OI/PI ratio represented the OB Risk Index (OBRI) to be compared with the CIMT modifications. **Results:** An average reduction of 22% in CH was achieved in the group of patients together with an increase in both OI and PI (16% and 39% respectively) with a significant improvement in OBRI from 2.6 to 1.7 (analysis of variance $P < .01$). A reduction of $>20\%$ in CIMT was obtained in 10 patients whereas in the 12 patients no modification of CIMT was detected, despite the same CH reduction (-55 ± 24.8 and -66 ± 27.1 mg/dL respectively; $P > .05$). Only those patients with a decrease in OBRI ≥ 0.8 showed a reduction in CIMT $>20\%$. **Conclusions:** In this preliminary study, a significant modification in CIMT was obtained with atorvastatin treatment only in those patients showing an improvement in the OB (OBRI > 0.8).

Keywords

atorvastatin, carotid media–intima thickness, oxidative balance, OBRI

Introduction

During the last 15 years, the epidemiological cardiovascular screening program of San Valentino has assessed more than 10 000 patients with careful recording of vascular risk factors and ultrasound scanning of both carotids and common bifurcations with a long-term follow-up.¹

The cholesterol (CH)-lowering therapy with statins is effective in reducing the CIMT²; however, some patients show the same progression of those receiving placebo.³ The concomitant reduction in high-sensitivity C-reactive protein (hs-CRP) and the high-density lipoprotein (HDL) increase have been considered as important cofactors for the efficacy of statins in decreasing CIMT.⁴

The oxidative stress (OS) reduction could be a determinant for the activity of some statins, although conflicting results have been reported about the capacity of statins as antioxidants. A reduction in plasmatic isoprostans has been described for simvastatin and rosuvastatin⁵; atorvastatin was found to reduce urinary isoprostans without significant change in malondialdehyde (MDA)⁶ suggesting that the protection is limited to the

membrane phospholipids and not to circulating fatty acids (free or bound to albumin) that can be oxidized down to alkenes. Other authors using hydroperoxides as a marker for oxidation found that atorvastatin was effective whereas pravastatin was inactive.⁷

These findings indicate that different markers of oxidation such as MDA (thiobarbituric acid reactive substances), isoprostans, carbonylated proteins, and oxidized DNA may represent

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different oxidation compartments and that statin's characteristics such as liposolubility and distribution volume may have impact on some of these compartments only.

Recently, the most common oxidative markers (hydroperoxides, oxidized DNA, carbonylated proteins, and isoprostanes) have been compared to show which one was the most sensible⁸ to detect a condition of OS determined in the healthy volunteers following a 1-week period of a standard diet with poor antioxidants. The result showed that more or less all the methods can pick up the OS condition, the only difference being the coefficient of variation (CV), which was between 40% and 60% for all the markers with the exclusion of hydroperoxides (reactive oxygen metabolites [d-ROMs] test) that was limited to 6% and 8% only. This low CV makes this test more suitable than the others for monitoring the OS condition. Furthermore, high levels of d-ROMs were shown to be an independent risk factor for cardiovascular events in patients⁹ with coronary artery disease and a significant correlation with hs-CRP was also shown by other authors.¹⁰

Another important factor is the antioxidant capacity. The balance between OS and antioxidant capacity can be defined as "oxidative balance" (OB) that until now was never correlated with the CIMT reduction.

The aim of our study was to determine the short-term (4 weeks) effects of a lipid-lowering treatment (atorvastatin) on CIMT progression in individuals within the San Valentino registry. These patients were lacking conventional risk factors other than hypercholesterolemia. The study evaluated the dynamic of CIMT in association with the modification of the OB. The oxidant offence was represented by the d-ROMs test whereas the antioxidant defense by plasma antioxidants test (PAT) and both tests were considered in relation to CH levels. A new index was proposed, the OB risk index (OBRI, which represents the ratio between the CH oxidation index (OI; d-ROMs test/CH levels) and the CH protection index (PI; PAT/CH levels) to be compared to the CIMT modifications.

Methods

Forty males with hypercholesterolemia were selected, in the age of 45 to 60 years who were found to have increased CIMT considering their age, in the absence of other diseases or conventional atherosclerotic risk factors, and not using any drugs.

The admission criteria were hypercholesterolemia (fasting CH > 240 mg/dL), triglycerides <170 mg/dL, body mass index <29 and <20 kg/m², and CIMT >0.1 mm/10 years of age (see later). Only patients with blood pressure <130 mm Hg (maximum) and <85 mm Hg (minimum) were admitted. The experience was conducted on 22 patients only who were not presenting carotid plaques (see later) and were not under treatment with any type of drug or food supplements. The remaining 18 patients were excluded because of the concomitant use of food supplements. After a preliminary discussion about the possible benefit of the hypolipidemic management, patients agreed to participate. In accordance with the declaration of Helsinki, patients who would directly and personally benefit

from the registry were considered and registered. There was no influence or pressure on registry patients from the observer or by their general physician.

Carotid Intima-Media Thickness and Ultrasound Scans

Carotid intima-media thickness was measured with ultrasound scanning methods that have been already described in detail.^{11,12} In brief, both carotids and femoral bifurcations were scanned using a PREIUS Elastasonographer (Hitachi Medical System, Singapore) with a linear-array transducer (10-14 vMhZ). The carotid and femoral arteries were imaged in transverse and longitudinal planes. Carotid artery examination included evaluation of the common carotid artery within 1.5 cm of the origin of carotid bulb, the carotid bulb itself, and the internal and external carotid arteries. The maximum CIMT was measured on the ultrasound image far wall by a mean of 5 measurements for each artery. Plaque was defined as a focal projection of at least 1.5 mm of the arterial wall into the lumen as defined according to previous work and confirmed for reproducibility.^{13,14} Patients with plaque were excluded. Carotid intima-media thickness was the only sign/marker of atherosclerosis.

The normal CIMT values were considered 0.1 mm/10 years of age: that is, patients aged 40 have CIMT <0.4 mm; patients aged 60 have CIMT <0.6 mm. This was an average in our population including patients without risk factors or significant disease or minimal risk condition and can be detected in some of 28% of the global population between 40 and 65 years.

Carotid Intima-Media Thickness Progression

Carotid intima-media thickness progression was the primary end point in terms of average of 5 measures ([left + right carotids]/2). The secondary end point was the relation of the CIMT progression to the OB. In previous studies, we had observed that to obtain a CIMT reduction both a decrease in CH and an improvement in OB are necessary. In cases with no modification of OB, the regression or reduced progression of CIMT or plaque growth may be minimal. The change in CIMT was determined by the sum of the score of the 2 carotid bifurcations expressed as a percentage decrease from the initial value.

Statin Administration

Atorvastatin was administered for a period of 4 weeks according to the following schedule of 10 mg for 2 weeks immediately followed by 20 mg for the other 2 weeks to be taken once a day.

Determination of Lipid

Blood sampling was done before treatment with atorvastatin (no more than 3 days before) and after 4 weeks of treatment. Blood was drawn from patients after overnight fasting in the quantity of 10 mL from the brachial vein part in EDTA tubes and 0.5 mL in heparinized microvettes. Serum CH and

triglycerides were measured using an enzymatic colorimetric assay and the common laboratory methods, and HDL was determined by phosphotungstic method. Low-density lipoprotein (LDL) was calculated using Friedewalds formula ($LDL = CH - [TG/5 + HDL]$).

Oxidative Balance

The blood collected in microvettes was centrifuged immediately and used for the OB determination in relation to total CH. The OB was based upon 2 different tests: plasma hydroperoxides (d-ROMs test)^{15,16} and PAT.¹⁷ The measures of oxidative and antioxidant variables were as follows: d-ROMs (1 Carr U = 0.08 mg/dL H₂O₂); PAT (1 Cor U = 1.4 μmol/L vitamin C). All the determinations were conducted using the FRAS-Evolvo C-OBRI System and relative disposable kits (H&D Srl Parma; Italy).

The total CH plasma level was taken as reference for the "oxidizable bulk" and 3 different indexes were calculated based upon the CH value: OI, corresponding to d-ROMs/CH ratio; PI, corresponding to PAT/CH ratio; and OB (OBRI), that is the OI-PI ratio. This last index was calculated according to the following formula:

$$(OI \times K_1/PI) \times (CH_t/200), \quad (a)$$

where K_1 is the factor needed to obtain a median of 1 (risk 1) when CH levels, d-ROMs test, and PAT are represented by normal average values, respectively: CH = 200 mg/dL; d-ROMs = 275 Carr U; PAT 2500 Cor U.

As a consequence, OBRI calculated on the average normal values will be equal to 1, only when $K_1 = 9.1$.

$$OBRI = OI \times 9.1/PI \times 275/200, \quad (b)$$

where $OI = 1.375$; $PI = 12.5$.

When CH_t (cholesterol level at the moment of the determination) will be 200 (the maximum normal level of total cholesterol), OBRI will be $= OI/PI \times 0.0455 \times CH_t$ ($OBRI = 1.375 \times 0.0455 \times 200 = 1$), OBRI will be $= OI/PI \times 0.0455 \times CH_t$. (c) is the formula currently applied for the calculation of OBRI.

In terms of oxidation, the total CH and not the fractions of LDL or HDL was considered. Oxidation of CH can be achieved in any lipoproteins, the only difference being the presence of paraoxonase in HDL that has a strong antioxidant activity.

Statistical Analysis

For all the data, the average and dispersion variable were calculated. The analysis of variance (ANOVA) was applied to determine the differences between the baseline values and those after 4 weeks of treatment. Correlation coefficient was also determined among all the variables.

Table 1. General Characteristics of the 22 Patients Treated With Atorvastatin.

Variable	Measure	Mean \pm SD
Age	Years	53 \pm 3.4
Sex	M	52.5 \pm 7.5
BMI	kg/m ²	25.2 \pm 0.32
Dosage	mg	10 mg for 2 weeks; 20 mg for 2 weeks
CIMT	mm	0.912 \pm 0.02
CH	mg/dL	282 \pm 22.8
LDL	mg/dL	211 \pm 16.9
TG	mg/dL	135 \pm 11.1

Abbreviations: BMI, body mass index; CIMT, carotid intima-media thickness; CH, cholesterol; LDL, low-density lipoprotein; TG, triglycerides; SD, standard deviation.

Compliance

No measurement of compliance was done since the CH reduction was considered as a suitable indicator of the statin intake.

Results

All the patients concluded the experience and their general characteristics are reported in Table 1. No side effects were reported and in every patient a reduction in CH was evident with an average decrease of 61 mg/dL (Table 2). The reduction of total CH in relation to the baseline levels was 22% ($P < .01$), whereas no significant modification of HDL and triglycerides were found. The hydroperoxides (d-ROMs test) in the 4 weeks period were reduced of about 10% ($P < .01$) and the antioxidant reserve was increased for about 8% ($P < .01$). Considering the oxidation in relation to the total CH, these modifications became more evident. The OI increase of about 15% (negative impact) corresponded to an increase in PI of 39% (positive impact). These indices were converging into an OBRI that showed a final average reduction of 34% improving from 2.56 to 1.68 ($P < .05$). The CIMT changes were found to be correlated with OBRI ($r = .790$, $P < .01$), indicating that the modifications of this is bound to the dynamic of CIMT.

The percentage of average modification of CIMT was $-12\% \pm 0.122\%$: in 10 patients of 22 showed a reduction between 18% and 31%, whereas in the remaining 12 patients the thickness was substantially unchanged (between +2% and -4%). The 10 patients with a significant reduction in CIMT were characterized by a decrease in OBRI of 1.2 ± 0.20 compared to 0.7 ± 0.21 in those patients with no improvement ($P < .05$).

Discussion

The weakness of this study is the short period of treatment and the limited number of patients recruited. Furthermore, the patients who were selected had hypercholesterolemia only with no other relevant symptoms. In other terms, the patient selection was very restrictive.

Table 2. Variables Related to Cholesterol Levels and Oxidative Balance Before (B) and After 4 Weeks (4w) of Treatment With Atorvastatin.

Variables	Measure	Period		Difference	Average, %	ANOVA
		B	4w	B-4w	B versus 4w	
CIMT	mm	0.91 ± 0.022	0.80 ± 0.108	0.11 ± 0.113	-12	<i>P</i> < .01
CH	mg/dL	282 ± 22.8	221 ± 21.5	61 ± 26.1	-22	<i>P</i> < .01
LDL	mg/dL	211 ± 16.9	152 ± 21.0	58 ± 23.2	-28	<i>P</i> < .01
HDL	mg/dL	43 ± 7.3	42 ± 5.4	1 ± 5.2	-3	<i>P</i> > .05
TG	mg/dL	135 ± 11.1	133 ± 12.1	2 ± 13.5	-2	<i>P</i> > .05
d-ROMs	Carr U	402 ± 8.5	363 ± 32.8	39 ± 34.3	-10	<i>P</i> < .01
PAT	Cor U	2012 ± 103.7	2182 ± 114.1	-171 ± 167.0	+8	<i>P</i> < .01
OI	Carr U/mg/dL	1.43 ± 0.125	1.66 ± 0.212	-0.22 ± 0.235	+16	<i>P</i> < .01
PI	Cor U/mg/dL	7.18 ± 0.613	9.96 ± 1.053	-2.79 ± 0.984	+39	<i>P</i> < .01
OBRI ^a		1.60 ± 0.089	1.05 ± 0.158	0.55 ± 0.198	-34	<i>P</i> < .01

Abbreviations: CIMT, carotid intima-media thickness; CH, cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; d-ROMs, reactive oxygen metabolites; PAT, plasma antioxidant test; OI, oxidative index; PI, protective index; OBRI, oxidative balance risk index.

^a The value was calculated according to the formula $OBRI = OI/PI \times 0.0455 \times CH_c$.

A larger number of patients should be followed for a longer period of time to confirm the validity of the results since those patients with no improvement in CIMT at short term could end up with a significant reduction at long term. Finally, the data obtained in this experience can be applied to atorvastatin only and not to all the other statins. Despite these limitations, some useful indication can be drawn from the improvement in OB as a variable related to the decrease in atheroprogession following the treatment with atorvastatin.

The first indication is that the OI may increase despite cholesterol reduction. This means that the lower levels of cholesterol (due to the 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibition) face the aggression of reactive species that can remain in a relative stoichiometric advantage. This means that a PI increase is necessary to overcome this relative oxidative threat, which can take place only in case the antioxidant system reacts promptly. In the present experience, for an average OI increase of 16%, the PI improves to 35%, witnessing a new equilibrium in favor of the antioxidant capacity.

The second important aspect is related to the OBRI reduction from 2.6 ± 0.22 to 1.7 ± 0.26 . The range of OBRI modification was between -0.5 and 1.5 and a possible cutoff of at least 0.8 seems to be necessary for a significant reduction in CIMT.

The cutoff OBRI of -0.80 was reached only in 10 patients. The average CH reduction in these patients was 55 ± 24.8 mg/dL, whereas in the remaining 12 patients it was 66 ± 27.1 mg/dL. Since this difference was not statistically significant (ANOVA *P* = .324), the CH reduction cannot be considered the only key for the improvement in CIMT. The average OBRI in the 10 patients was -0.73 ± 0.110 , whereas in the other 12 patients it was -0.41 ± 0.120 only (ANOVA *P* < .001).

This clearly indicates that the CH reduction per se is not the only determinant for the limitation of CIMT and other factors such as the OB improvement have to be considered. This last objective can be obtained with different methods, the most obvious being the diet rich in vegetables (and fruit) which increase the availability of circulating antioxidants. The

physical activity also is known to improve the enzymatic antioxidant capacity.

The reason of a more consistent improvement in the OB in 10 patients of 22 is unknown and probably due to a healthier diet and physical activity. Unfortunately, we do not have such data to confirm the hypothesis.

The antioxidant activity of atorvastatin has already been reported by some authors,⁶ together with the plasmatic measurement of aminothiol levels in terms of cysteine, cystine, GSH (reduced glutathione), and the relative Nernst equation (cysteine/cystine and GSSG/GSH₂ (ratio oxidized/reduced glutathione) respectively), which mirror the antioxidant capacity. These authors showed a significant reduction in the OS (less evident than in our study), but the antioxidant capacity was not modified since it was measured on the basis of single antioxidants, instead of measuring the total antioxidant capacity. The separation of antioxidants dilutes the total effect that is the combination of the activity of many compounds.

The oxidation of CH contained in lipoproteins is limited by the liposoluble antioxidants (tocopherols α and δ) that are located on the membranes. Once these antioxidants have donated the reductant capacity, they have to be regenerated by the circulating antioxidants. These are represented not only by thiols but also by vitamin C, uric acid, polyphenols, and many other entities (eg, bilirubin and transferrin). This "body" of antioxidants can be determined by PAT or alternatively by TAS,¹⁸ being the 2 methods significantly correlated¹⁹ (internal data).

In conclusion, the data of the present experience are suggestive of the importance of the OB considering both the oxidant and the antioxidant compounds in relation to CH levels that can be determined in terms of OBRI.

The results should be confirmed in larger trials, with different statins administered for a longer period of time. One may also consider that therapies associated with statins can have impact on the OB. The OBRI determination seems to be important to monitor the therapy of cardiovascular diseases.

Authors' Note

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