Variations in Echogenicity in Carotid and Femoral Atherosclerotic Plaques with Pycnogenol + Centella Asiatica Supplementation

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Abstract

This registry study evaluated echogenicity of carotid–femoral plaques in asymptomatic subjects with increased oxidative stress and risk factors (mild hypertension, hypercholesterolemia). Supplementation with the combination Pycnogenol–CA (centella asiatica) on the echogenicity of plaques was assessed at 6 months (79 subjects). A standard management (SM) plan was used in all subjects (control of risk factors, lifestyle changes); 36 subjects used the supplements +SM; 43 SM only. The groups were comparable. High-resolution ultrasound evaluated echogenicity and plaque structure. Pycnogenol (150 mg/day) and CA (Centellicum, 450 mg/day) were used.

At 6 months, cholesterol was reduced ($p < 0.05$) in both groups (difference between groups not significant). At 6 months, plasma free radicals were decreased with the supplements (17.64%; $p < 0.05$; vs $<2\%$ in controls). The plaque stability index increased from 11.22;2.3 to 22.4;1.1 ($p < 0.05$) with the supplements; no significant changes were seen in controls. Plaque echogenicity (% of “whiter” component in images) increased with supplementation from 16.7;1.7% to 34.2;2% ($p < 0.05$); no variations were observed in controls. The maximum plaque height decreased ($p < 0.05$) with the supplements. No significant variations were observed in controls. Plaque length was decreased ($p < 0.05$) in the supplement group with no changes in controls. The number of plaques (carotid, femoral bifurcations) decreased with supplementation; no significant changes were observed in controls. No adverse events, tolerability problems, or variations in routine blood tests were recorded. The combination Pycnogenol–CA appears to improve echogenicity and stability of complex plaques in 6 months.

Keywords

- plaques
- atherosclerosis
- Pycnogenol
- Centella asiatica
- echogenicity
- oxidative stress
- cardiovascular events

Cardiovascular events, including acute coronary syndromes (myocardial infarction, angina, sudden ischemic death) and cerebral infarctions can be lethal or cause severe medical and social consequences. If not fatal, they can result in decreased quality of life, social degradation, and increased human and medical costs. Cardiovascular events may occur following the sudden rupture of atherosclerotic plaques.^{1–3} To prevent these types of events, several types of prophylactic plans aim at plaque stabilization. Many plaques, defined unstable,

may be made less unpredictable by reducing the plaque's mainly lipid/thrombotic, “soft” structure and by thickening the fibrous matrix covering the plaque.

Large-scale trials have shown that the risk of cardiovascular events and mortality can be reduced by cholesterol-lowering therapy with statins.^{1–4} Statins have a range of pleiotropic effects that are independent from their main action of lowering lipids and cholesterol levels. These effects include improvements in endothelial function, decreased

smooth muscle cell migration and proliferation, and decreased transport of oxidized lipids into the arterial wall eventually determining plaque stabilization.^{5,6} A specific product (cerivastatin) has shown significant pleiotropic effects at low concentrations (in comparison with other statins), and it has been used in a model of plaque stabilization study.^{5–9} In a specific plaque study, Kurata et al⁹ used cerivastatin to stabilize carotid plaques with good effects on the fibrous matrix that was increased making plaques more dense and stable: however, the product used for that study was considered dangerous as it exposed patients to side effects.^{10–12} During postmarketing surveillance, 52 deaths were reported in patients treated with cerivastatin, mainly for rhabdomyolysis and renal failure. Cerivastatin also induced myopathy.¹²

However, there is still an important need for plaque stabilization that may be independent from statin management. Pycnogenol, an anti-inflammatory, antioxidant, and mildly antithrombotic agent derived from the bark of a specific French pine (Horphag, Geneva) is a highly standardized and safe supplement; it has been used in several clinical, vascular conditions.^{13–17} Centella asiatica (CA) has been shown to produce a decrease of inflammation, scarring, and keloid formation (i.e., after burns)^{18–20} with a significant collagen modulating action and a very high safety profile. The recent combination of these two supplements has been used in subjects with atherosclerotic plaques to stop progression and to make plaques smaller and more stable.²¹

The aim of this pilot registry study was the evaluation of the echogenicity and potential stability of carotid plaques in asymptomatic, low-risk subjects, with increased oxidative stress and the effects of the supplementation with the combination Pycnogenol–CA on the improvement of echogenicity – and therefore plaque stability – in a 6-month registry.

Patients

This registry study included asymptomatic patients with:

- moderate hypercholesterolemia (total cholesterol level > 220 mg/dL) managed without drug therapy (diet and lifestyle management) for at least 6 months
- stable mild hypertension managed with a minimum dose of ace inhibitors (Ramipril 10 mg/day)
- one dishomogeneous atherosclerotic plaque at the bifurcation of the carotid and/or femoral arteries with differential densities within its structure (→ Fig. 1) and a combination of high and low echogenicity elements (white black and gray on ultrasound);
- the plaque caused an average of 50% stenosis (range 43–63.2%) as shown by high-resolution ultrasound.

Subjects were available to the follow-up of, at least 6 months.

All patients also had an elevated oxidative stress.

Patients with a history of significant cardiovascular disease (associated to severe events), including coronary artery events (myocardial infarctions, documented, short-range, or

Fig. 1 A complex plaque, such as the plaque presented in this image (diagram of an ultrasound scan at the carotid bifurcation), was the target of this registry study. The lowest density areas are the black areas (same density of blood); the white areas (higher echogenicity or density, corresponding to a high content of functional, structural collagen) are the elements of the arteries less prone to disruption. A lipidic–thrombotic core is (schematically) shown in gray scale.

unstable angina) or using other drugs, were excluded from the registry study.

Concomitant medications or supplements were not used or were exclusion criteria (i.e., patients with diabetes or metabolic disorder, requiring treatment or diet were excluded).

Informed consent was obtained from all subjects.

Supplementation. The subjects were supplemented with an association of Pycnogenol and CA (Centellicum). Both these highly standardized supplements were used orally, for 6 months. Pycnogenol was used at the dose of 150 mg/day (in three caps) and Centellicum at the dose of 450 mg/day (in two caps).

Supplement studies. These registry studies^{22–24} define the field of activity of pharma-standard supplements and their possible preventive, preclinical applications. The best fields of application for supplements are preclinical, borderline applications or the supplementary management of risk conditions. Supplements, unless there are specific claims, are not generally used for treatment of clinical conditions; they may be used to manage “minor” medical problems. Supplement studies produce supplementary data to be compared with “background” historical data (i.e., based on the best available management for comparable subjects) or to other management plans. In this study the supplement combination was used according to the following rules:

- The use of the supplement was suggested to the evaluation subjects; it was not prescribed but indicated as an option, possibly capable of improving the management of the risk condition.
- The supplement was only used on top of what was considered the “standard” or “best-management/care” available, for that condition, according to international guidelines.

- The use of the supplement should not have interfered with any other treatment, management, or preventive measure.
- Time: the period of follow-up was considered variable, according to the needs and availability of the patients or registry subjects. The observation period is therefore variable, not prefixed. Ideally, the supplement should be used as long as needed to see results or changes.
- The type of evaluation for these studies is always a registry. The evaluation of the compliance concerning the use of the supplement is a significant value indicating how many subjects are willing to use the supplements. In these supplement studies, there is no defined group allocation, no randomization organized by the investigators. Subjects decide – on the basis of an initial briefing – the management group they want to join including the control (nonsupplement) group. No placebo is used.

Standard management. Asymptomatic subjects with plaques, unless there is a specific condition requiring treatment may be managed by a hypolipidic and hypocaloric diet, NaCl restriction, regular exercise, and weight control with progressive elimination of potential risk factors (i.e., smoking).

Oxidative stress; plasma free radicals. A secondary endpoint in this registry was the evaluation of changes in plasma-free radicals to measure changes in oxidative stress. This was measured by the d-ROM test (a colorimetric method using the Diacron kit – FRAS, H&D, St, Langhirano, Parma, Italy) from a drop of blood.²⁴ The results were expressed in Carr units. A value > 330 Carr units for patients of this age and distribution indicate increased oxidative stress.²⁴

Centella asiatica (Centellicum, Horphag, Geneva), generally modulates the scarring process, and it has been shown to be effective in modulating the growth and remodeling of plaques at the carotid and femoral level.^{18,19,21} **Pycnogenol** has also been shown to modulate inflammation and oxidative stress and, in atherosclerotic studies, it has been effective in improving echogenicity and stability of atherosclerotic plaques.^{13–17,21}

Assessments. In each subject, the largest plaque at the carotid and femoral bifurcations was assessed using a color duplex, high-resolution ultrasound imaging system (Preirus, Hitachi, Japan). Images were obtained with a linear probe (14 Mhz).^{25,26} Longitudinal (plaque longer axis) digital ultrasound images were obtained at the beginning and at the end of the study. The highest resolution (b-mode) images were “normalized” into a standard gray scale using as a reference a 0 value for full white (the highest echogenicity in the image, corresponding to the density of the adventitia) to a 100 value (full black in the image, considered the lowest possible echogenicity, corresponding to the density of blood) as the black reference.^{26,27}

This “normalization” method and the evaluation of echogenicity in arterial plaques have been described and validated in previous publications by our group^{26–30} and defined in details.³¹

Fig. 2 Diagram of a high-resolution ultrasound scan of the carotid bifurcation. The target of the study was to improve the echogenicity of the plaque structure (in plaques considered with a low level of echogenicity and therefore with a limited stability). Also the fibrous cap in the initial image is very thin; it may appear segmented, non-continuous. This element may predispose the plaque to rupture into the lumen with consequent embolization or local thrombosis. In this registry, the structure of the plaque was improved by becoming more echogenic (or white on ultrasound). The incorporation of collagen into the plaque could be the effector of this improvement. Also the fibrous cap could be seen as more echogenic, “white,” more continuous.

Using these techniques and the methods described by Kurata in the model study,⁹ the cholesterol esters in plaques appear as low-intensity regions (toward black, almost the same density of blood) and the fibrous (echogenic) matrix is shown as a high-intensity region (white) on the image. The percentage of the total plaque section (at the maximum longitudinal ultrasound section on scans) appearing on the scan as white or high-intensity regions was defined as the *plaque stability index*⁹; higher values indicate a greater stability of the plaque due to increased presence of collagen and elastic matrix.

Several previous studies^{25–30} have indicated that the presence of dark components in plaques (corresponding by density to lipids, blood, clots, thrombi) tends – more frequently – to produce embolization or induce local thrombosis. These plaques with low-echogenicity, “black” areas are generally less stable (► Fig. 2).

The *total number of plaques* at the carotid and femoral bifurcations was also recorded with high-resolution scans.^{25,32,33}

Total cholesterol level and other laboratory values (hematocrit, ESR, hemoglobin thyroid, liver and renal function tests, and serum protein) were measured at inclusion and at 6 months. They were normal (excluding cholesterol) at inclusion.

Tolerability and compliance to the supplements were recorded.

Severe calcifications in the plaque or arteries do not allow a clear ultrasound visualization, therefore, subjects with important calcification (producing acoustic shadows) were excluded.

One *single target plaque* (the less echogenic and the more dishomogeneous one in four bifurcations, or the one with the largest low-density components) was considered as the main “target” plaque either at the carotid or femoral bifurcations for the evaluation of echogenicity in time.

The *gray scale median* (according to the method described by Sabetai^{26,29,30} was used to evaluate the global echogenicity of the single plaques and their variations in time).

The surface of these plaques (at the intima-blood flow contact zone) was regular with a visible fibrous cap that is generally associated to a lower risk of rupture and embolization.³¹

No antiplatelet agent was used during the registry period.

Statistical analysis. Data were analyzed using nonparametric tests; measurements are reported as means $\pm$ SD. Statistical significance was defined as $p < 0.05$.

The relative changes in echogenicity, plaque stability index, plaque number, gray-scale median, and plasma free radicals were all considered nonparametric and were tested using χ^2 test and ANOVA (with Bonferroni correction). A numerosity of at least 20 subjects per group (SM vs SM + supplementation) was considered necessary (on the basis of previous studies) to achieve a statistical difference in 3 months.

Results

The subjects followed in this registry study, divided into two groups, at 6 months were comparable (**►Table 1**).

Cholesterol values were reduced ($p < 0.05$) in both groups at 6 months (as a consequence of management). The total cholesterol decrease at the end of the study (16.8% with the supplement vs 18.7% with SM) in both groups was a consequence of the management plan. The difference between the two groups was not significant. As indicated in the model study (Kurata, 9) the variation of cholesterol for 6 months could not be associated to important changes in plaque characteristics which appear to be independent from lipid lowering.

Also blood pressure (**►Table 1**, well controlled before and at the end of the registry) and triglycerides changes were minimal at 6 months in the two groups.

Plasma free radicals were significantly decreased in the supplement group (17.64% on average; $p < 0.05$) in comparison with minimal (<2%) differences in controls using only the SM plan. The correlation between a decreased oxidative stress and an important improvement in plaque stability (and the associated no-growth) needs to be explored in more specific studies.

The *plaque stability index* increased significantly from an average 11.22;2.3 at inclusion to 22.4;1.1 at six months ($p < 0.05$) in the supplement group while no significant changes were seen in the comparable, SM group (**►Table 2**).

At 6 months, *plaque echogenicity* (indicating the percent of the “white” component in the digital images) was also increased in the supplement group from 16.7;1.7% to 34.4;2% ($p < 0.05$). No significant variations were observed in controls.

The observed increase in echogenicity 17.5 in 6 months (equivalent to 2.9 per month) may be projected to a theoretical increase in echogenicity possibly >30% in a year.

However, even a limited increase in echogenicity may produce a significant increase in stability and a clinically

Table 1 Details of patients and variations in 6 months

		Control SM	Py + CA Supplementation	Group Difference (P)
Completing				
Subjects	Number	43 (females 12)	36 (15)	
	Age	57.4;3.2	58;2.2	ns
Previous smokers (all quit before inclusion)		36/43	28/36	ns
Lost		4	4	ns
Chol (mg/dL)	Incl	238;13	229;12.2	ns
	6 Mon	197.4;12.3	192;13.2	ns
Blood PR (mmHg) (max/min)	Incl	133/92	134/90	ns
	6 Mon	131/91	120/87	ns
Plasma FR radicals (Carr units)	Incl	399;29	391;21	ns
	6 mon	385.2;22	328.3;19	<0.05

important reduction in possible embolic or thrombotic cardiovascular events.

The *maximum plaque height* decreased significantly ($p < 0.05$) following intake of the supplements. No significant variations were observed in controls. *Maximum plaque length* was also significantly decreased ($p < 0.05$) in the supplement group with no significant changes in controls. The difference between the two groups was significant ($p < 0.05$).

The *total number of plaques* detected at the carotid and femoral bifurcations decreased in 6 months with the supplement association; however, the decrease was clinically minimal; no significant changes were observed in controls.

Arterial flow characteristics (percent stenosis) remained unchanged ($<5\%$).

No supplement-related adverse events, tolerability problems, or abnormal variations in routine (hematocrit, kidney and liver function test, and metabolic parameters) laboratory test values were recorded during the registry and at the end of the study (► **Table 2**).

Discussion

Cardiovascular events linked to plaque fragility, disruption, and to their complications (mainly thrombosis or embolization) can be theoretically (in a long period of time) prevented by controlling classical risk factors and, specifically, by cholesterol-lowering therapy.^{4–6} But cardiovascular events also occur in patients with normal lipid levels; therefore, lowering cholesterol and lipids is not the only significant action to consider in the prevention of cardiovascular events.

Localized inflammatory reactions in blood vessels during the onset of atherosclerosis occur continuously and complicate plaque structures. Substances and forces injuring the vascular endothelium (oxidized, low-density lipoprotein and glycoprotein, shear stress, microtrauma, calcifications etc.) may all alter vascular endothelial function,^{34,35} vascular wall perfusion, and plaque evolution. Inflammatory reactions of different types may occur. Some may include monocyte

adherence and endothelial migration; some involve the differentiation and evolution into macrophages with the formation of foam cells: the resulting low-density plaques may have a very thin and fragile fibrous cap that may easily break, producing events.

In the Kurata model study,⁹ cerivastatin administration both lowered total cholesterol levels and made carotid artery plaques more stable and dense. The increase in plaque density was considered separate from the cholesterol management (considering the short period of observation).

A recent study³⁶ indicates that in the presence of a higher oxidative stress accumulation in plaques their growth is increased. Therefore, the use of an antioxidant supplement with a strong, reliable, consistent action may help to control the local oxidation of lipoproteins and the growth of plaques.³⁶ The observation that plaque stabilization and even regression may be independent from cholesterol lowering but may be associated to the levels of oxidative stress may indicate new ways to control plaque growth that it is basically a form of irregular, localized scarring.³⁷

The combination of the highly standardized supplements Pycnogenol and CA appears to be effective in controlling atherosclerosis progression in asymptomatic subjects^{16,18} even in the presence of risk factors (cholesterol, hypertension, increased oxidative stress) and in controlling atherosclerosis progression to symptomatic stages (eventually reducing the number of cardiovascular events linked to the presence of plaques)²¹ with a very good tolerability and safety record. The effect on plaque echogenicity can be seen (by ultrasound) in only 3 months. The anti-inflammatory, antioxidant, and antiplatelet activities of Pycnogenol (pine bark-derived and therefore broadly overlapping for its multiple activities to aspirin, also, originally, bark-derived) are associated with a very important level of tolerability that cannot be obtained with aspirin. Inhibitory effects on inflammatory reactions – related to the inhibition of macrophage evolution into foam cells with production of matrix metalloproteinases (MMPs)³⁸ – can be contrasted by Pycnogenol

Table 2 Plaque factor variations

Plaque characteristics		SM	SM + Pycno-CA	
Stability index (%)	Incl	11.1;1.7	11.22;2.3	ns
	6 Mon	11.2;1.3	22.4;1.1	*
Echogenicity (0 = black; 100 = white)	Incl	17.3;1.4	16.7;1.7	ns
	6 Mon	19.4;1.3	34.2;2	*
Max plaque height (mm)	Incl	3.23;0.2	3.34;0.3	ns
	6 Mon	3.29;0.7	2.86;0.2	*
Max PLAQ length (mm)	Incl	12.2;1.1	13.2;1	ns
	6 Mon	13.3;1.2	12;0.7	*
Total number of plaques longer than 1 cm or thicker than 20 mm (carotid + femoral bifurcations)	Incl	4.2;1.1	4.1;0.8	ns
	6 Mon	4.2;0.7	3.6;1	*

* $p < 0.05$.

supplementation. The modulation of the collagen structural core, the white, echogenic components, in the type of plaques described in the present registry study, is facilitated by CA. Increased level of collagen makes plaques more stable and produce an improvement of the covering surface (at the blood–plaque interactive layers), thickening the thin fibrous layer with a matrix “protecting” the plaque from dynamic blood interactions and rupture.

In a study in progress, the echogenicity of the fibrous layers can be seen increasing with the association Pycnogenol and CA, and becoming more visible in weeks.

This registry, exploratory study indicates that a significant increase in plaque density and stabilization may be achieved with the combination of Pycnogenol and CA. The problem of plaque instability is common and requires medical solutions, easy to apply to most patients.^{39,40} The supplementation can be obtained with self-medication, without prescription and without dangers of side effects. It could be a significant, important, preventive option for many patients who do not have an increase in blood lipids but have a plaque, potentially at risk of rupture and prone to cause cardiovascular events. Pycnogenol may also help by improving endothelial function⁴¹; this supplement has a mild antiplatelet aggregation activity.⁴² It may be therefore considered a “global”, important form of natural, early prevention for asymptomatic atherosclerosis.

Supplements need to be in standardized preparations (with manufacturing standards, consistency, and defined dosages) to be used and to have a reliable, replicable effect.⁴³

Further supplement or clinical studies need to be organized including a larger number of patients (i.e., including hypertensive, diabetics, and higher-risk subjects) and for longer periods, also considering how plaque stabilization may affect the occurrence of cardiovascular events in defined periods of time.

Conflict of Interest
None.

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