Plasma cGMP and Large Artery Remodeling in Asymptomatic Men

Marie-Aude Devynck, Alain Simon, Marie-Gabrielle Pernollet, Gilles Chironi, Jérôme Gariepy, Francine Rendu, Jaime Levenson

Abstract—cGMP regulates vascular smooth muscle tone and arterial wall response to proliferative signals. We determined plasma cGMP and carotid artery intima-media thickness (IMT) and diameter in 84 asymptomatic men submitted to investigation of their cardiovascular risk profiles. Plasma cGMP was positively associated with IMT (P<0.01) and diameter (P<0.05), independently of coexisting risk factors. These associations were reinforced in the subgroup of subjects with high-sensitivity C-reactive protein level or multiple atherosclerotic plaques. A positive relationship existed between diameter and IMT (P<0.01) and disappeared after cGMP adjustment. This suggests a link between cGMP pathway and arterial wall geometry that is revealed by vascular injury conditions and may participate in early large artery remodeling. (Hypertension. 2004;44:1-5.)

Key Words: atherosclerosis • nitric oxide • endothelium • arteries • remodeling • cyclic GMP

Release of cGMP in response to natriuretic peptides or NO regulates vascular smooth muscle tone and arterial wall changes to proliferative signals. Although the vasodilator effect of cGMP is well documented, the conditions under which it exerts antiatherogenic or proatherogenic effects remain unclear.1–5 Because of its effects on vascular relaxation and proliferation, cGMP may influence control of diameter and wall thickness of arteries. The question thus arises whether cGMP measurement in plasma in man may add new insights to the understanding of mechanisms that regulate large artery geometry6 at the initial stage of arterial disease, in which a phenomenon of remodeling occurs.7 To this end, we measured plasma cGMP and concomitantly assessed carotid intima-media thickness (IMT) and diameter by ultrasound in men.

Methods

Subjects

The study population constituted 84 consecutive asymptomatic subjects addressed to our Center of Cardiovascular Prevention by occupational health medicine doctors in the framework of a program of cardiovascular prevention described previously,8 aiming to determine their cardiovascular risk factor profile (Table 1). A condition of selection was that they were free of any treatment (for hypertension, hypercholesterolemia, or diabetes), free of any cardiovascular disease (stroke, coronary heart disease, heart failure, or peripheral vascular disease), and that they had undergone plasma cGMP measurement after written informed consent (Table 1). Body mass index was calculated as the ratio of weight to height squared. Resting brachial blood pressure was measured by sphygmomanometer, and hypertension was defined as blood pressure at ≥140/90 mm Hg.8

Fasting blood lipids and glucose were measured by enzymatic methods (after precipitation of LDL and VLDL for HDL measurement), and hypercholesterolemia was defined by total cholesterol of ≥5.2 mmol/L, and diabetes by blood glucose of ≥7 mmol/L.9 Plasma high-sensitivity C-reactive protein (hs-CRP) was measured by high-sensitivity immunoassay.7 Current smoking was defined by daily consumption of ≥1 cigarette for ≥3 months. We estimated the risk of coronary heart disease by entering age, male sex, systolic blood pressure, total and HDL-cholesterol, and presence or absence of smoking into the Framingham Model equations.8 A total of 33% of subjects were hypertensive with average value of blood pressure of 148/92 mm Hg; 64% had hypercholesterolemia with total cholesterol level on average of 6.3 mmol/L, and 42% were current smokers (Table 1). Because of the presence of multiple risk factors, 48% of the study population had a Framingham 10-year risk of ≥10%, and 36% were carriers of diffuse subclinical atherosclerosis constituted of plaques at 2 or 3 extracoronary sites (see below) that attests the presence of high cardiovascular risk8 (Table 1).

Plasma cGMP Measurement

Blood was collected on citrate anticoagulated tubes and immediately centrifuged. Plasma was deproteinized by addition of 25 volumes of absolute ethanol and boiling before centrifugation. After evaporation, the sample was dissolved in 0.05 mol/L Na-acetate buffer, pH 6.2. cGMP was acetylated and measured by radioimmunoassay (Perkin–Elmer), with a variation coefficient averaging 6.4% between repeated measures in the same blood sample and 10% between 2 repeated examinations in the same subject. Distribution of cGMP values was not normal (Figure 1). Circulating nitrite concentration was also measured simultaneously with plasma cGMP by the Griess method10 in 20 additional blood samples.

Ultrasound Measurements

Far wall IMT and lumen diameter were measured along ≥1 cm in both common carotid arteries, 2 to 3 cm upstream of bifurcation, by
Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.5 (8.4)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.6 (3.3)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>129.0 (16.5)/82.2 (10.2)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.66 (1.04)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.10 (0.39–2.22)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.13 (0.60–4.00)</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.3 (0.6)</td>
</tr>
<tr>
<td>Framingham risk at 10 years (%)</td>
<td>9.9 (0.5–30.5)</td>
</tr>
</tbody>
</table>

Risk factors (n, %)
- Hypercholesterolemia: 54 (64%)
- Hypertension: 28 (33%)
- Smoking: 35 (42%)
- Increased risk at 10 years (>10%): 40 (48%)
- hs-CRP (mg/L): 1.22 (0.18–19.9)
- Plasma cGMP (nmol/L): 2.08 (0.24–16)
- Carotid artery IMT (mm): 11.7 (2.9)

Values are No. (n, %), mean (SD), or median (minimum and maximum).

High-resolution ultrasound (ATL 5000; Philips), and computerized image analysis, with a variation coefficient between 2 repeated examinations averaging 3.6% and 2.4%, respectively. The presence of intracoronary plaque at 3 main extracoronary arterial sites (extracranial carotid arteries, abdominal aorta, and femoral arteries) was also detected according to a procedure described and validated previously. Plaque was detected by high-resolution ultrasound (ATL 5000; Philips), using a probe with an operating frequency of 5 to 12 MHz for carotid and femoral investigations and of 5 MHz for the aorta. Plaque was defined as a focalized encroachment into lumen by >1.5 mm. When ≥1 plaque was found in 1 site, the site was considered diseased, with 94% to 100% agreement between 2 repeated examinations. The number of disease sites was coded as grade 0, 1, 2, or 3 and classified into 0 to 1 or 2 to 3 disease sites (Table 1).

Statistical Analysis
Log transformation was used for parameters with skewed distribution. Univariate regressions were performed by least square method. Multivariate regressions were performed with general linear model. We tested interactions between parameters by entering the products of independent parameters 2 by 2 in the model.

Results
No association existed either between cGMP and clinical and biological parameters or between cGMP and atherosclerotic plaques, as assessed by the number of disease sites. In contrast, cGMP was associated positively with IMT (P<0.01), diameter (P<0.05), and CSA-IMT (P<0.01; Figure 2), and associations persisted after adjustment for Framingham risk (P<0.01, P<0.05, and P<0.001, respectively). Plasma cGMP was also positively associated with circulating nitrite concentration (r=0.62; P<0.01). Finally, a positive association existed between diameter and IMT (r=0.31l P<0.01), which persisted after adjustment for Framingham risk (P=0.02) but disappeared when cGMP was added as covariate (P=0.07).

Multivariate analysis of carotid parameters on cGMP and hs-CRP (taken as a continuous variable) showed that IMT, diameter, and CSA-IMT were associated with cGMP, hs-CRP, and the product of cGMP with hs-CRP (P<0.05). This last finding indicated an interaction between cGMP and hs-CRP, which allowed the association of carotid parameters and cGMP to be analyzed separately in 2 subgroups defined by the category of hs-CRP level (higher or lower hs-CRP according to its median of distribution, 1.22 mg/L). The risk-adjusted associations of cGMP with IMT, diameter, and CSA-IMT were significant in the subgroup with higher hs-CRP (P<0.01, P<0.05, and P<0.01, respectively), although insignificant in that with lower hs-CRP (Table 2; Figure 3). Multivariate analysis of carotid parameters on cGMP and the number of disease sites (classified into 0 to 1 or 2 to 3 disease sites) showed that IMT, diameter, and CSA-IMT were associated with cGMP (P<0.05), the number of disease sites (P<0.05), and the product of cGMP with the number of disease sites (P<0.05) except diameter, the association of which with the product almost reached the significance level (P=0.07). The interaction between cGMP and the number of disease sites allowed the association of carotid parameters and cGMP to be analyzed separately in 2 subgroups defined by the number (0 to 1 or 2 to 3) of disease sites. The risk-adjusted associations of cGMP with IMT, diameter, and CSA-IMT were significant in the subgroup
with 2 to 3 disease sites ($P<0.01$, $P<0.05$, and $P<0.01$, respectively), although insignificant in that with 0 to 1 disease sites (Table 2; Figure 3).

**Discussion**

Because cGMP, through its effects on vascular relaxation and proliferation, may influence control of diameter and wall thickness of arteries, its measurement in plasma concomitantly with the assessment of carotid IMT and diameter may be relevant for a better understanding of the mechanisms that regulate large artery geometry at the initial stage of arterial disease. Using this rationale, we found an association of plasma cGMP with carotid artery IMT and diameter in a population of asymptomatic men for whom a substantial proportion can be considered at intermediate or high cardiovascular risk because of the presence of traditional risk factor(s), increased Framingham risk score, or the presence of diffuse subclinical atherosclerotic plaques. The association of cGMP and carotid artery geometry is original and suggests a link between a signaling product of NO/natriuretic peptide pathways and large artery wall phenotypes. Indeed, cGMP has been considered a reflection of natriuretic peptides in patients with cardiac dysfunction and as an indicator of NO synthase activity in healthy subjects. Because our study subjects were free of cardiac dysfunction, cGMP was more likely a signaling product of NO pathway than an effect of natriuretic peptides. The positive association found between cGMP and circulating nitrites reinforces this possibility. Furthermore, the possibility that circulating cGMP reflects cGMP concentration in the arterial wall is supported by studies showing that cGMP egression from endothelial and vascular smooth muscle cells was attributable to active transport system at a rate proportional to its intracellular concentration.

IMT and lumen diameter were measured only in the common carotid artery because we have shown, in agreement with others, that this site of measure has the best precision and reproducibility rates. The positive association of cGMP with diameter agrees with the vasodilator effect of cGMP. In contrast, that of cGMP with IMT was at odds with the common belief that cGMP restrains vascular smooth muscle cell proliferation. The administration of NO donor as nitrate treatment might have been interesting for assessing the time course of cGMP effects on IMT. Although the effects of acute nitrate administration cannot be expected to mimic the conditions of the present work, the investigation of a group under chronic nitrate treatment would have been more appropriate. Unfortunately, patients under chronic nitrate treatment have clinical coronary artery disease and therefore receive concomitantly other drugs such as statins, angiotensin-converting enzyme inhibitors, or $\beta$-blockers, which are likely to have a confounding influence on the relationship between cGMP and IMT.

Besides IMT and lumen diameter, we have also detected the presence of intrusive plaques at 3 main extracoronary sites (extracranial carotids, abdominal aorta, and femoral) according to a procedure validated previously. It is interesting to remark that contrary to IMT, there was no significant association between cGMP and atherosclerotic plaques defined as the number of disease sites. A possible explanation is that plaque and IMT do not represent the same type of arterial

**Table 2. Relationships of Carotid Artery Geometry With cGMP According to hs-CRP Level or No. of Disease Sites**

<table>
<thead>
<tr>
<th>hs-CRP levels</th>
<th>IMT</th>
<th>Diameter</th>
<th>CSA-IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower (n=42)</td>
<td>0.027 (0.026)</td>
<td>0.09 (0.25)</td>
<td>0.72 (0.79)</td>
</tr>
<tr>
<td>Higher (n=42)</td>
<td>0.095 (0.036)†</td>
<td>0.57 (0.22)*</td>
<td>3.00 (0.89)†</td>
</tr>
<tr>
<td>No. of disease sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1 (n=54)</td>
<td>0.017 (0.023)</td>
<td>0.17 (0.20)</td>
<td>0.68 (0.68)</td>
</tr>
<tr>
<td>2–3 (n=30)</td>
<td>0.161 (0.045)‡</td>
<td>0.63 (0.28)*</td>
<td>4.50 (1.13)‡</td>
</tr>
</tbody>
</table>

Values are slopes (SE) adjusted for Framingham risk.

* $P<0.05$; † $P<0.01$; ‡ $P<0.001$. 

![Figure 2. Relationships of cGMP with carotid artery IMT, diameter, and CSA-IMT.](http://hyper.ahajournals.org/Downloadedfrom)
wall change. IMT, as measured in the common carotid artery, is a preintrusive thickening of large artery wall that is not synonymous with atherosclerosis. Indeed, increased IMT may be attributable to increased intimal thickness in relation to early atheroma, to increased medial thickness in relation to a nonatherosclerotic hypertrophic process, or to both phenomena.\textsuperscript{16} In contrast, plaque that is a focalized intrusive structure inside the vascular lumen is synonymous with atheroma. Therefore, it is suggested that cGMP is related to early preintrusive change of large artery wall as assessed by IMT but not to advanced atherosclerotic plaque. In addition IMT, measured in the common carotid far wall, cannot be considered a precursor of plaque because this site is generally free of atherosclerotic lesion that develops preferentially in areas of bifurcation or curvature in relation to low shear stress phenomena induced by these geometric features.\textsuperscript{16}

Mechanisms that may account for the association of cGMP with IMT are not clear. It may be hypothesized that vascular injury associated with atherogenic factors plays a role by increasing cGMP and IMT concomitantly. Indeed, it has been reported that vascular injury stimulates inducible NO synthase and therefore may increase cGMP.\textsuperscript{3,4} It also stimulates growth factors and may therefore increase IMT.\textsuperscript{3,4} This hypothesis is supported by the fact that the relationship between cGMP and IMT was revealed by subclinical inflammation (higher hs-CRP level) or by diffuse atherosclerosis (2 or 3 sites with plaques), which both reflect a proatherogenic state. An alternative hypothesis is that hypertrophic smooth muscle shifts its phenotype from contractile to secretory and hence increases cGMP production as described previously.\textsuperscript{3} It cannot also be excluded that the cGMP-dependent signaling pathway may have proatherogenic or proliferative effects. Indeed, it was shown recently that postnatal ablation of the cGMP-dependent protein kinase I (cGKI) in murine smooth muscle cells attenuates development of smooth muscle cell–derived plaque and atherosclerotic lesions.\textsuperscript{3} Recent data in hypercholesterolemic rabbits show that early arterial alteration occurs with reduced activity of cGKI despite a marked increase in inducible NO synthase expression and total NO production.\textsuperscript{17}

The associations between cGMP and carotid IMT on one hand, and between cGMP and carotid lumen diameter on the other, suggest that cGMP might be related to large artery remodeling. Indeed, carotid remodeling can be considered the mutual adaptation of wall thickness and lumen diameter, leading diameter to increase when artery wall thickens, as reported previously.\textsuperscript{7} This remodeling is reflected in this work by a positive association of carotid IMT with carotid lumen diameter. The possibility that cGMP may play a role in this remodeling is supported by its associations with both carotid IMT and lumen diameter and by the fact that the association between carotid IMT and carotid lumen diameter loses its statistical significance after adjustment for cGMP. Nevertheless, the cross-sectional nature of our study is a limitation that does not allow the absolute demonstration of a causal role of cGMP in vascular remodeling to be provided.

**Perspectives**

Our findings suggest that the NO/cGMP pathway participates independently in large artery early remodeling that occurs at the initial stage of arterial disease, possibly via stimulation of inducible NO synthase in response to systemic atherogenic or inflammatory conditions. This brings new insight to the mechanisms of vascular remodeling and supports recent findings on the proatherogenic role for cGMP-dependent protein kinase.\textsuperscript{3} This also suggests that plasma cGMP measurement might have a clinical impact and could be a candidate marker for prevention of cardiovascular disease. However, these preliminary results need to be confirmed by experimental or clinical studies comprising an intervention...
arm (NO donor, NO oxidation inhibitor, or phosphodiesterase inhibitor) capable of producing temporal changes in cGMP and large artery structure.

Acknowledgments

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References

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