Plasma Heme Oxygenase-1 Levels and Carotid Atherosclerosis

Yoshimi Kishimoto, PhD; Kenji Sasaki, MD; Emi Saita, PhD; Hanako Niki, MD; Reiko Ohmori, PhD; Kazuo Kondo, MD; Yukihiko Momiyama, MD

Background and Purpose—Heme oxygenase-1 (HO-1) catalyzes the oxidation of heme to generate carbon monoxide, biliverdin, and iron. Because these products have antiatherogenic properties, HO-1 may play a protective role against atherosclerosis. However, plasma HO-1 levels in patients with carotid atherosclerosis have not been reported.

Methods—We investigated plasma HO-1 levels by ELISA in 136 subjects (age, 66±9 years) undergoing carotid ultrasonography.

Results—Of the 136 study subjects, carotid plaque was found in 61 subjects (45%). Compared with 75 subjects without plaque, 61 with plaque were older and predominantly male (P<0.05). Plasma HO-1 levels were higher in subjects with plaque than in those without plaque (median, 0.56 versus 0.44 ng/mL; P<0.05). The percentage of subjects with HO-1 level >0.50 ng/mL was higher in subjects with plaque than without plaque (66% versus 44%; P<0.025). In multivariate analysis, HO-1 level was a significant factor for carotid plaque independent of atherosclerotic risk factors. Odds ratio for plaque was 2.33 (95% CI, 1.15–4.75) for HO-1 level >0.50 ng/mL.

Conclusions—Plasma HO-1 levels were high in subjects with carotid plaques, probably reflecting a protective response against carotid atherosclerosis. (Stroke. 2018;49:2230-2232. DOI: 10.1161/STROKEAHA.118.022256.)

Key Words: atherosclerosis ■ biliverdine ■ carbon monoxide ■ carotid artery diseases ■ heme oxygenase-1

Heme oxygenase-1 (HO-1) is an intracellular enzyme that catalyzes the oxidation of heme to generate carbon monoxide, biliverdin, and ferrous iron. Mainly because of the degradation of pro-oxidant heme, the generation of anti-oxidant biliverdin, and the production of vasodilator carbon monoxide, HO-1 is considered to have protective properties against atherosclerosis.1 However, HO-1 expression was observed throughout the progression of atherosclerosis from early fatty streaks to advanced lesion.2 HO-1 expression in endothelial and smooth muscle cells was upregulated on the exposures to reactive oxygen species and oxidized low-density lipoprotein.3 In apolipoprotein E–deficient mice, a lack of HO-1 accelerated atherosclerosis,3 whereas HO-1 induction reduced atherosclerosis in low-density lipoprotein receptor knockout mice.4 Moreover, adenovirus-mediated gene transfer of HO-1 reduced atherosclerosis.5 Therefore, HO-1 expression in atherosclerotic lesions is considered to be a protective response against the progression of atherosclerosis. Recently, elevated blood levels of HO-1 were reported in some chronic diseases, such as diabetes mellitus.6 Although HO-1 is recognized to be released into plasma from leukocytes, macrophages, smooth muscle cells, and endothelial cells that are activated or damaged by oxidative stress or inflammation,7,8 few studies have examined plasma HO-1 levels in patients with atherosclerotic diseases. Idriess et al7 reported plasma HO-1 levels to be higher in 70 patients with coronary artery disease than in 50 controls. However, no study reported plasma HO-1 levels in patients with carotid plaques. Therefore, we examined the association between plasma HO-1 levels and carotid atherosclerosis using carotid ultrasonography.

Methods

Study Subjects

The data that support the findings of this study are available from the corresponding author on reasonable request. We investigated plasma HO-1 levels in 136 consecutive subjects (mean age, 66±9 years) who underwent carotid ultrasonography as well as an ankle-brachial index test for medical check-up to evaluate atherosclerosis at Tokyo Medical Center. Our study was approved by institutional ethics committee (R16-012), and written informed consent was obtained (Methods in the online-only Data Supplement).

Carotid Ultrasonography

Both right and left carotid arteries were evaluated from longitudinal and transverse views of common, bifurcation, and internal carotid arteries by high-resolution B-mode ultrasonography. Intima-media thickness was measured using a computer-assisted method. Plaque was defined as a focal wall thickening ≥1.5 mm or ≥50% of the...
surrounding intima-media thickness. The extent of plaque was evaluated as a total plaque score which was calculated as the sum of points (range, 0–12) of all 6 segments (Methods in the online-only Data Supplement).

**Measurements of Plasma HO-1 and High-Sensitivity C-Reactive Protein Levels**

Plasma HO-1 levels were measured by ELISA with a commercially available kit (Human HO-1 ELISA Kit; Enzo Life Sciences Inc, Farmingdale). High-sensitivity C-reactive protein levels were measured by BNII nephelometer (Dade Behring, Tokyo, Japan; Methods in the online-only Data Supplement).

**Statistical Analysis**

Because the distributions of measured HO-1 levels were considered to be highly skewed and to be nonparametric variables by Shapiro-Wilk test, results were presented as the median value. Correlations were evaluated by Spearman rank correlation test. To determine the cutoff point of HO-1 levels for plaque, a relative cumulative frequency distribution curve was created, and the optimum cutoff point was 0.50 ng/mL. A P<0.05 was considered statistically significant (Methods in the online-only Data Supplement).

**Results**

Of the 136 study subjects, carotid plaque was found in 61 (45%), of whom 31 had a plaque score of 1, 21 had a score of 2, 6 had a score of 3, and 3 had a score of 4. Compared with 75 subjects without plaque, 61 with plaque were older (69±8 versus 64±8 years) and predominantly male (P<0.05; Table 1). Plasma high-sensitivity C-reactive protein levels tended to be higher in subjects with plaque than in those without plaque (median, 0.39 versus 0.34 mg/L), but this difference did not reach statistical significance. However, HO-1 levels were significantly higher in subjects with plaque than without plaque (median, 0.56 versus 0.44 ng/mL; P<0.05; Figure). A stepwise increase in HO-1 levels was found depending on plaque score: 0.44 ng/mL in score=0 (n=75), 0.51 ng/mL in score=1 (n=31), and 0.70 ng/mL in score ≥2 (n=30; P<0.02; Figure). Moreover, HO-1 levels significantly correlated with plaque score (r=0.23; P<0.01) but not with mean intima-media thickness or age. The percentage of subjects with HO-1 level >0.50 ng/mL was higher in subjects with plaque than without plaque (66% versus 44%; P<0.025). In multivariate analysis, HO-1 level was a significant factor for carotid plaque independent of atherosclerotic risk factors. Odds ratio for plaque was 2.33 (95% CI, 1.15–4.75; P<0.025) for HO-1 level >0.50 ng/mL (Table 2).

**Discussion**

In the present study, plasma HO-1 levels were significantly higher in subjects with carotid plaque than without plaque and were a significant factor for carotid plaque independent of atherosclerotic risk factors.

Because HO-1 catalyzes the oxidation of heme to generate carbon monoxide, biliverdin, and iron, HO-1 has protective properties against atherosclerosis. In endothelial and smooth muscles cells, HO-1 expression was markedly upregulated by oxidized low-density lipoprotein, and HO-1 overexpression was reported in atherosclerotic lesions. Hence, HO-1 overexpression in atherosclerotic lesions is considered to be a protective response against the progression of atherosclerosis. Regarding plasma HO-1 levels in patients with atherosclerotic diseases, plasma HO-1 levels in patients with coronary artery disease were reported to be high. However, no study has reported plasma...
HO-1 levels in patients with carotid atherosclerosis. Cheng et al\textsuperscript{11} reported HO-1 expression in 112 carotid endarterectomy samples to be higher in vulnerable plaques than in stable plaques, but Ameriso et al\textsuperscript{12} reported HO-1 expression in 48 carotid endarterectomy samples to be less prevalent in symptomatic patients than in asymptomatic patients. Our study first reported that plasma HO-1 levels were higher in subjects with carotid plaque than in those without carotid plaque. Thus, patients with carotid atherosclerosis as well as those with coronary artery disease have high levels of HO-1 in plasma. High plasma HO-1 levels may reflect an increased oxidative stress condition and may be aimed at protecting from the progression of atherosclerosis. However, further studies are needed to elucidate the mechanism and role of high plasma HO-1 levels on atherosclerosis.

In conclusion, plasma HO-1 levels were found to be high in subjects with carotid plaque and were a significant factor associated with carotid plaque independent of atherosclerotic risk factors. High levels of plasma HO-1 in subjects with carotid plaque may reflect a protective response against atherosclerosis.

**Sources of Funding**

This study was supported by a grant from Honjo International Scholarship Foundation. Financial funding was also provided by Bayer Yakuhin Ltd and Pfizer Japan Inc; however, these sponsors had no role in the design, analysis, or interpretation of our study.

**Disclosures**

None.

---

**Table 2. Factors Associated With Carotid Plaque**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&gt;65 y)</td>
<td>2.22</td>
<td>1.10–4.49</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HO-1 level (&gt;0.50 ng/mL)</td>
<td>2.33</td>
<td>1.15–4.75</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

The dependent variable was the presence of carotid plaque. The analysis included age (>65 years), sex, hypertension, hyperlipidemia, statin use, diabetes mellitus, smoking, and HO-1 level (>0.50 ng/mL). HO-1 indicates heme oxygenase-1.

---

**References**