Haem oxygenase-1: a target for dietary antioxidants

R.M. Ogborne, S.A. Rushworth, C.A. Charalambos and M.A. O’Connell
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, U.K.

Abstract
HO-1 (haem oxygenase-1) is a stress-response enzyme involved in the catabolism of haem. In animal models, it plays a key protective role in vascular disease. HO-1 has anti-inflammatory effects in macrophages and is induced by a range of stimuli, including antioxidants, in various cell types. As dietary antioxidants are considered to be beneficial in vascular disease, their protective effects may occur through induction of HO-1. Emerging evidence suggests that a range of dietary and other naturally occurring antioxidants stimulate HO-1 expression in various cell types, although regulation by these compounds has not been investigated in detail. These studies suggest that HO-1 may be a target for dietary therapy in vascular disease.

HO-1 (haem oxygenase-1)
HO (haem oxygenase) catalyses the rate-limiting step in the catabolism of haem to biliverdin, a reaction that liberates equimolar amounts of free iron and carbon monoxide. Biliverdin is subsequently converted into bilirubin, a powerful antioxidant, by biliverdin reductase. Free iron is sequestered by the storage protein ferritin. Three isoforms of HO have been identified which are cytosolic proteins that anchor to the endoplasmic reticulum at the C-terminus. HO-1, HO-2 and HO-3 arise from distinct genes and have varying tissue distributions and regulatory pathways. Although HO-2 and HO-3 are constitutively expressed, HO-1 is inducible in many cell types. The primary inducer of HO-1 expression is its substrate, haem [1].

HO-1 and vascular disease
Recent developments in the study of atherosclerosis have considered the underlying inflammatory process and the role of antioxidative responses and exogenous antioxidants. Given that haem itself is a pro-oxidant and bilirubin an antioxidant, HO-1 may play a role in protecting the vessel wall. In the low-density lipoprotein receptor knockout model of atherosclerosis, mice given a high-fat diet had significantly increased expression of HO-1 in atherosclerotic lesions, particularly foam cells and macrophages, in comparison with controls [2]. Mice deficient in both HO-1 and apolipoprotein E had a more rapid and progressive atherosclerotic lesion formation [3]. In addition, human HO-1 deficiency results in severe persistent endothelial damage with increased thrombomodulin and von Willebrand factor [4]. These studies suggest that HO-1 is important in the vascular system.

Dietary antioxidants induce HO-1
HO-1 is induced by a wide range of stimuli apart from haem, including various antioxidants. Pyrrolidine dithiocarbamate induces HO-1 expression in rat vascular-smooth-muscle cells [5]. t-Butylhydroquinone stimulates HO-1 in murine fibroblasts and macrophages [6]. As these antioxidants induce HO-1 expression in various cell types, dietary antioxidants, which are reputed to be beneficial in vascular disease, may also augment HO-1 expression. Table 1 summarizes some recent results available in the literature regarding the induction of HO-1 by dietary antioxidants in animal cells. We have also included data obtained from other experiments performed in our laboratory, which studied human cells and the induction of HO-1 by dietary antioxidants that inhibit vascular gene expression.

ALA (α-lipoic acid) is a potent thiol-containing antioxidant found in spinach, broccoli and tomatoes. ALA has been reported to inhibit cytokine and nitric oxide production in monocytes [7]. We have found that ALA dose-dependently induces HO-1 expression in THP-1 human monocytic cells (Figure 1). Curcumin, a polyphenol with antioxidant properties, is derived from the spice turmeric. It inhibits NF-κB (nuclear factor κB)-regulated gene expression, cyclo-oxygenase activity and lipid peroxidation [8] and, therefore, may have beneficial effects in vascular disease. Curcumin induces HO-1 expression in various cell types, including bovine endothelial cells and rat macrophages [8–10]. We have found recently that curcumin induces HO-1 expression in primary human monocytes and THP-1 cells (Figure 1). Grapes and red wine are a rich source of the antioxidant resveratrol, which has anti-inflammatory effects on vascular cells and inhibits NF-κB-mediated gene expression. Resveratrol has been reported to induce HO-1 expression in murine neuronal cells at low doses (5 μM) [11].
**Table 1 | Dietary antioxidants that induce HO-1 expression**

THP-1, acute monocytic leukaemia; PC12, pheochromocytoma; LLC-PK1, renal epithelial cells.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Diet source</th>
<th>Dose (µM)</th>
<th>Cell type</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>Broccoli, spinach, tomatoes</td>
<td>250–1000</td>
<td>THP-1</td>
<td>Human</td>
<td>Unpublished results*</td>
</tr>
<tr>
<td>Cafestol and kahweol (synergy)</td>
<td>Coffee</td>
<td>3–18</td>
<td>Primary hepatocytes</td>
<td>Rat</td>
<td>[13]</td>
</tr>
<tr>
<td>Carnosol</td>
<td>Rosemary</td>
<td>10</td>
<td>PC12</td>
<td>Rat</td>
<td>[14]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Turmeric</td>
<td>30</td>
<td>BAEC</td>
<td>Bovine</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>LLC-PK1</td>
<td>Porcine</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15–30</td>
<td>Astrocytes</td>
<td>Rat</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–15</td>
<td>THP-1</td>
<td>Human</td>
<td>Unpublished results*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Primary monocytes</td>
<td>Human</td>
<td>Unpublished results*</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Grape</td>
<td>5–100</td>
<td>Neurons</td>
<td>Murine</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>THP-1</td>
<td>Human</td>
<td>Unpublished results*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>HUVEC</td>
<td>Human</td>
<td>Unpublished results*</td>
</tr>
<tr>
<td>Selenium (as Ebselen)</td>
<td>Cereals, fish</td>
<td>3–30</td>
<td>Primary cardiac myocytes</td>
<td>Rat</td>
<td>[15]</td>
</tr>
<tr>
<td>Sulphoraphane</td>
<td>Broccoli, sprouts</td>
<td>0.05–1</td>
<td>Aortic smooth-muscle cells</td>
<td>Rat</td>
<td>[12]</td>
</tr>
</tbody>
</table>

* Unpublished results from experiments performed in our laboratory.

**Figure 1 | ALA and curcumin induce HO-1 mRNA expression in THP-1 monocytic cells**

In ALA experiments, THP-1 cells were unstimulated (white bars) or stimulated with 0.25 mM (light grey bars), 0.5 mM (dark grey bars) or 1 mM (black bars) ALA for 4 h. In curcumin experiments, THP-1 cells were unstimulated (white bars) or stimulated with 1 µM (light grey bars), 5 µM (dark grey bars) or 15 µM (black bars) curcumin for 4 h. mRNA was extracted and relative quantitative mRNA expression determined by real-time PCR with HO-1 expression normalized against 18 S ribosomal mRNA expression using the comparative cycle threshold method.

In contrast, 5 µM resveratrol had no effect on HO-1 activity in rat astrocytes and, at higher doses, inhibited the enzyme activity [8]. It was found from experiments performed in our laboratory that 25 µM resveratrol only weakly induced HO-1 expression in human monocytes and endothelial cells (S.A. Rushworth, R.M. Ogborne and M.A. O’Connell, unpublished work). Other dietary antioxidants have also been reported to induce HO-1 expression, including the coffee diterpenes cafestol and kahweol, carnosol and low doses of sulphoraphane (0.1 µM) [12–14]. In summary, these studies indicate that several structurally diverse dietary antioxidants induce HO-1 expression in a variety of vascular cell types.

**Regulation of HO-1 transcription**

HO-1 gene expression is tightly regulated at the transcriptional level. The HO-1 promoter contains several transcriptional regulatory elements that respond to redox-sensitive transcription factors, including Nrf2 (NF-E2 related factor 2). Nrf2 is ubiquitously expressed in human cells and is a member of the basic leucine zipper family of transcription factors. Under basal conditions, Nrf2 resides in the cytoplasm bound to its inhibitor protein, Keap-1, an actin-binding protein. On stimulation, Nrf2 translocates to the nucleus and binds to a DNA sequence in target genes known as the antioxidant response element [6].

The antioxidant 1,4-butylhydroquinone requires Nrf2 to activate HO-1 expression [6]. Emerging evidence suggests that dietary antioxidants may also induce HO-1 through Nrf2. Curcumin induced Nrf2-dependent HO-1 promoter activity in porcine renal epithelial cells. This required the p38 mitogen-activated protein kinase [10]. In PC12 cells, carnosol-induced HO-1 transcription also required Nrf2. In contrast with the previous study, phosphatidylinositol-3-kinase, but not p38, appears to be important in this pathway [14]. The mechanisms by which other dietary antioxidants activate Nrf2 are unclear.

**Conclusion**

HO-1 is protective in models of atherosclerosis. Emerging evidence suggests that dietary antioxidants up-regulate HO-1 in vascular cells and this may be a mechanism for their beneficial effects in this disease. Further studies are required to investigate the effects of other dietary antioxidants and examine the signalling pathways involved in HO-1 expression.

**References**


Received 20 July 2004