ORAL DISEASES

Oral Diseases (2011) 17, 252–257 doi:10.1111/j.1601-0825.2010.01732.x

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REVIEW ARTICLE

The heme oxygenase system and oral diseases

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Heme oxygenase (HO) system catabolizes heme into three products: carbon monoxide (CO), biliverdin/bilirubin and free iron, which consists of three forms identified to date: the oxidative stress-inducible protein HO-1 and the constitutive isozymes HO-2 and HO-3. HO has been involved in many physiological and pathophysiological processes, ranging from Alzheimer's disease to cancer. The interest in HO system by scientists and clinicians involved with the oral and maxillofacial region is fairly recent, and few papers currently cited on HO relate to diseases in this anatomical area. This review will focus on the current understanding of the physiological significance of HO-1 induction and its possible roles in the oral diseases studied to date. The implications for possible therapeutic manipulation of HO are also discussed.

Oral Diseases (2011) **17**, 252–257

Keywords: heme oxygenase; review; oral disease; cancer

Introduction

The heme oxygenase (HO) system is the most effective mechanism in the cell for cleaving heme into carbon monoxide (CO), biliverdin and free iron. In the course of this catalysis, biliverdin is rapidly converted to bilirubin and the iron is reutilized for maintenance of iron homeostasis and gene regulation (Maines, 2004). Three HO isoforms have been identified to date: HO-1, HO-2, and HO-3, among which the isoforms 1 and 2 are the best known. HO-2 is constitutively and most highly expressed in neuronal tissues contributing to cell homeostasis. The only chemical inducer of HO-2 identified to date is adrenal glucocorticoids (Gcs). HO-1, also referred to as heat shock protein-32 (HSP32), is an inducible enzyme and expressed relatively low in most tissues, which can be strongly induced in response to cellular stress and diverse oxidative stimuli (Maines, 1997). HO-3, which is described in the rat brain, has no activity and is not expressed in humans (McCoubrey *et al*, 1997).

HO-1 and HO-2 are both viewed as playing a major role in heme breakdown. All products of HO activity are now suspected to be biologically active, which metabolic pathway is involved in a wide variety of physiological and pathophysiological processes (Figure 1) (Ryter *et al*, 2006). Recent studies have indicated that HO system may be involved in different oral diseases as shown in Table 1.

HO-derived CO and its effects

Almost all CO produced *in vivo* comes from the degradation of heme by HO. Depending on the cell type, CO can activate one or both of two key signaling pathways, through which CO involves in numerous physiological and pathophysiological conditions (Figure 1). One of the pathways is soluble guanylate cyclase (sGC)/cyclic guanosine monophosphate (cGMP). For example, HO-derived CO activates the sGC, stimulating the production of the intracellular second messenger, cGMP. The sGC/cGMP pathway has been implicated in mediating the effects of CO on vascular contractility, the inhibition of smooth muscle proliferation and neurotransmission (Verma *et al*, 1993; Duckers *et al*, 2001). Moreover, CO has been shown to inhibit platelet aggregation and to prevent apoptosis in endothelial cells (Brouard *et al*, 2002) and fibroblasts (Petrache *et al*, 2000) through the pathway. Another one is p38 mitogen-activated protein kinase (MAPK) pathway, through which CO can in large measure mediate the anti-inflammatory actions (Yachie *et al*, 1999; Otterbein *et al*, 2000; Lee and Chau, 2002). For example, Otterbein *et al* demonstrate that exogenous CO delivered at low concentrations differentially and selectively inhibits lipopolysaccharide (LPS)-induced pro-inflammatory cytokines including tumor necrosis factor-α and increases production of the anti-inflammatory cytokine interleukin-10 (Otterbein *et al*, 2000; Lee and Chau,

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Received 10 January 2010; revised 4 June 2010; accepted 12 June 2010

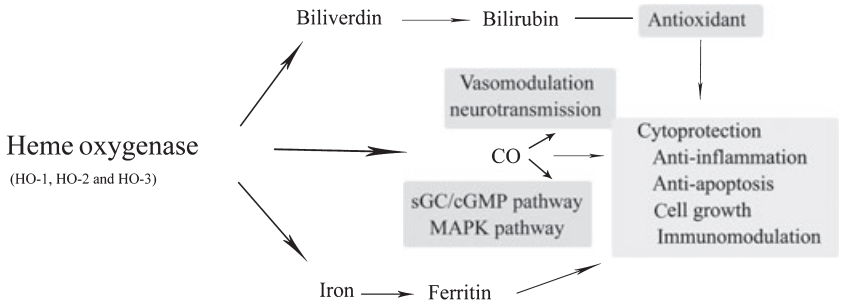

Figure 1 Schematic demonstration of the HO system and its biologic activities

Table 1 Heme oxygenase expression in the oral and maxillofacial region

Isoform	*Tissue or cells*	*Inducing agent/diseases*	*References*
HO-1	Basal cells of oral mucosa	Normal	Lee *et al* (2008b), Tsai *et al* (2009
HO-1	Salivary gland acini and ducts	Normal	Lo *et al* (2005)
HO-2	Salivary gland acini and ducts	Normal	Lo *et al* (2005)
HO-1	Fibroblasts, endothelial cells, inflammatory cells	Areca quid/oral submucous fibrosis	Tsai *et al* (2009)
HO-1	Human keratinocyte cell	Arecoline *in vitro*	Thangjam and Kondaiah (2009)
HO-1	Basal cells and superficial layers of oral mucosa	Carcinoma in situ	Lee *et al* (2008b)
HO-1	Odontoblasts, endothelial cells	Carbamide peroxide	Anderson *et al* (1999)
HO-1	Basal layers of epithelium, inflammatory cells, fibroblasts	Cigarette	Chang *et al* (2005)
HO-1	Pulp cells	Hydrogen peroxide	Min *et al* (2008a)
HO-1	Immortalized human oral keratinocytes	Hydrogen peroxide	Min *et al* (2008c)
HO-1	Pulp cells	Interleukin-1alpha	Min *et al* (2006)
HO-1	Periodontal ligament cells	Lipopolysaccharide	Jeong *et al* (2009)
HO-1	Odontoblast-like cells, pulp fibroblasts	Mineral trioxide aggregate or calcium hydroxide	Min *et al* (2008b)
HO-1	Periodontal ligament cells	Nicotine	Jeong *et al* (2009)
HO-1	Malignant oral keratinocytes	Nicotine *in vitro*	Lee *et al* (2008a)
HO-1	Malignant oral keratinocytes	Nitric oxide *in vitro*	Lee *et al* (2007a)
HO-1	Gingival fibroblasts	Nicotine *in vitro*	Chang *et al* (2005)
HO-1	Basal cells of oral mucosa	Oral epithelial dysplasia	Lee *et al*, 2008b)
HO-1	Basal and prickle cell layers	Oral lichen planus	Lee *et al*, 2008c)
HO-1	Cancer cells	Oral squamous cell carcinoma	Lee *et al*, 2008b)
HO-1	Tumour cells	Parotid pleomorphic adenomas	Lo *et al*, 2005)
HO-2	Tumour cells	Parotid pleomorphic adenomas	Lo *et al* (2005)
HO-1	H400 oral epithelial cells	Porphyromonas gingivalis	Milward *et al* (2007)
HO-1	Periodontal ligament cells	Substance P	Lee *et al* (2007b)

2002). Moreover, Chin and Otterbein (2009) recently point out that CO has been ascribed an additional novel role as a host defense molecule agent against microbes (bactericidal agent) because of its property of anti-inflammation.

HO-derived biliverdin or bilirubin and its effects

HO catalyzes the rate-limiting step in heme degradation to biliverdin. Biliverdin is, in turn, converted into bilirubin by biliverdin reductase at the expense of NADPH. Biliverdin and bilirubin are reducing species and hence potent antioxidants (Figure 1) (Stocker *et al*, 1987; Abraham and Kappas, 2005). Several studies have demonstrated that the administration of biliverdin and/or bilirubin is potently cytoprotective in a variety of pathophysiological events, including ischemia-reperfusion injury, transplant rejection and inflammatory bowel disease (Clark *et al*, 2000; Fondevila *et al*, 2004; Nakao *et al*, 2004). Also, high-normal or slightly elevated levels of bilirubin decreased risk for early familial coronary artery disease (Hopkins *et al*, 1996) and a low prevalence of ischemic heart disease (Vitek *et al*, 2002). The antiatherogenic properties of bilirubin may include inhibitory effects against low-density lipoprotein (LDL) oxidation and scavenging of oxygen radicals (Ishikawa *et al*, 2001). In addition, bilirubin is now also known to modulate immune effector functions and suppress inflammatory response (Willis *et al*, 1996).

HO-derived $Fe^{2+}$and its effects

Fe^{2+}, which is also a product of heme degradation, upregulates an iron-transporter pump that removes

intracellular Fe^{2+} from the cell (Ferris *et al*, 1999) and induces the expression of ferritin, an iron storage protein (Balla *et al*, 1992). Expression of ferritin is originally reported to protect endothelial cell against oxidant damage *in vitro* (Balla *et al*, 1992). In addition, over-expression of H-ferritin (heavy chain ferritin) has also been shown to protect cultured endothelial cells from undergoing apoptosis and protected livers from transplant-associated ischemia-reperfusion injury (Berberat *et al*, 2003). This protection may be attributed to ferritin, which limits the generation of free radicals by binding free Fe^{2+} that would otherwise participate in the Fenton reaction to promote the generation of reactive oxygen species (Otterbein *et al*, 2003).

HO and cytoprotection

HO-1 is involved in a variety of regulatory and protective cellular mechanisms as a stress-responsive protein. Min *et al* (2008b) have reported that HO-1 is expressed in the odontoblast-like cells and pulp fibroblasts in human pulp capping with different materials. Furthermore, they also show that induction of HO-1 by hydrogen peroxide (H_2O_2) in pulp cells plays a protective role against the cytotoxic effects of H_2O_2 (Min *et al*, 2008a). According to the same group, HO-1 may play a role in the cytoprotection of human pulp cells treated with interleukin-1alpha in the early inflammatory reaction (Min *et al*, 2006). Others have shown that expression of HO-1 is increased in coronal odontoblasts and endothelial cells when bleaching vital teeth with 10% carbamide peroxide (Anderson *et al*, 1999). These results indicate that HO-1 expression provide an important cellular response in many different cell types of oral cavity, which protects cells against oxidative damage. The increased level of HO expression may serve as a first protective environment against acute oxidative and inflammatory insults.

Oral mucosal inflammatory diseases

HO-1 is expressed in the basal cells of normal oral mucosa (Lee *et al*, 2008b; Tsai *et al*, 2009). Few studies have investigated HO expression in oral mucosal inflammatory diseases. Tsai *et al* demonstrate that HO-1 expression is significantly increased in oral submucous fibrosis (OSF) from areca quid chewers, and arecoline, as an oxidative insult, may be responsible for the up-regulated HO-1 expression *in vivo* (Tsai *et al*, 2009). Moreover, Lee *et al* find that there is an elevation of HO-1 expression in oral lichen planus (Lee *et al*, 2008c), which is considered as a cell-mediated, chronic inflammatory disease of unknown etiology. Recent studies have also demonstrated that HO-1 and ferritin light chain in human keratinocyte cells are upregulated by arecoline *in vitro* (Thangjam and Kondaiah, 2009). HO-1 is known as a stress-inducible protein and functions as an antioxidant enzyme, which is commonly regarded as a potent anti-inflammatory enzyme (Jozkowicz *et al*, 2007). Thus, the upregulation of HO-1 in oral mucosa is implicated in endogenous mechanism against inflammation.

Periodontal disease

There have been few studies which have investigated the role of HO in periodontal disease. Some authors assess expression of HO-1 in smoking-associated periodontal disease *in vivo* and *in vitro*. They find that HO-1 expression is significantly higher in the basal layers of epithelium, inflammatory cells, and fibroblasts in cigarette smokers and human gingival fibroblasts exposed to nicotine (Chang *et al*, 2005). A similar study done by Jeong *et al* (2009) demonstrate that HO-1 is involved in the anti-inflammatory activity of Apigenin in human periodontal ligament (hPDL) cells treated with nicotine- and lipopolysaccharide (LPS). Moreover, Milward *et al* (2007) mimic the biological processes associated with periodontitis by the effects of Porphyromonas gingivalis (PG, a periodontopathogen) on cultured H400 oral epithelial cells and find that HO-1 is 21.1-fold up-regulated. Lee *et al* (2007b) analyze expression of HO-1 following the effects of substance P (SP) on human periodontal ligament cells and demonstrate that HO-1 is implicated in the development of periodontitis or inflammation during orthodontic tooth movement.

Salivary gland diseases

There have been few published reports in this area and only one paper to date has investigated HO expression in salivary gland diseases. This study investigates the expression HO-1 and HO-2 in human parotid pleomorphic adenomas. They find that normal salivary gland acini and ducts display significantly stronger immunoreactivity for HO-2 compared to tumour cells. The positive staining for HO-1 is seen in normal salivary ducts and in pleomorphic adenomas showing ductal differentiation (Lo *et al*, 2005). The pleomorphic adenoma is by far the most common benign salivary gland tumor. Although its pathogenesis is not clear, the findings suggest that HO may be implicated in the pathogenesis of salivary pleomorphic adenomas (Lo *et al*, 2005).

HO and oral carcinoma

Increased oxidative damage associated with disturbances in antioxidant defense system have been implicated in the pathogenesis of oral cancer. For example, in oral squamous cell carcinoma (OSCC) patients, there is an increase oxidative stress associated with a deficient antioxidant defense (Gokul *et al*, 2010). HO-1 is known as an oxidative stress responsive protein, which has been proposed to protect cells against oxidative damage. HO-1 exhibits cytoprotective effects not only in many normal cell types, which we have discussed above, but also in cancer cells. Studies show that HO-1 plays a major role in mediating cytoprotection and iron homeostasis against nitric oxide toxicity (Lee *et al*, 2007a) and nicotine (Lee *et al*, 2008a) in immortalized and

malignant oral keratinocytes *in vitro*. Furthermore, HO-1 expression is significantly upregulated in OSCC patients (Lee *et al*, 2008b,d).

Oxidative damage can cause DNA base alterations, including the activation of oncogenes or inactivation of tumour suppressor genes and make it possible for transition from a normal somatic cell to a cancer cell. These changes allow the cell to escape normal control mechanisms and lead to carcinogenesis (Brennan *et al*, 2003), in an early phase of which up-regulation of HO-1 play a part in cytoprotection mechanism in the oral mucosa. It is shown in a clinical finding, in which the level of up-regulation of HO-1 in oral epithelial dysplasia (a premalignant oral lesion) is higher than in OSCC and correlated with the degree of epithelial dysplasia (Lee *et al*, 2008b). However, the increasing and/or persistent oxidative stimuli might make HO-1 keep in a higher level. The higher activity of HO-1 seems to facilitate tumor growth (Jozkowicz *et al*, 2007).

HO-1 can directly affect cell viability by antiapoptotic effects. This property is shown in fibroblasts (Petrache *et al*, 2000) and endothelial cells (Brouard *et al*, 2000). Apparently, protection against apoptosis plays an important role in the cancer-supportive environment (Schwartsburd, 2003). HO-1 is upregulated in HPV-immortalized human oral keratinocytes (IHOK) and oral cancer cells (HN4) treated by H_2O_2. The increased levels of HO-1 could be responsible for limiting the progression of H_2O_2 induced cellular apoptosis (Min *et al*, 2008c). Nevertheless, some investigators show that HO-1 can not protect human OSCC-3 cells from Physalis angulata-initiated apoptosis, which is attenuated through inhibition of the proteins expression of HO-1 (Lee *et al*, 2009). In a similar study, Lee *et al* (2010) report that isoliquiritigenin 2'-methyl ether (a chloroform extract of Caesalpinia sappan L.) induces apoptosis in oral cancer cells and have shown the anti-oral cancer effects, the mechanism in which upregulation of HO-1 is involved via different signal pathways. Obviously, there are some contradictory findings concerning the antiapoptotic effects of HO-1. A possible explanation of the inconsistencies would be that the studies are performed in different tumor cells, which are subjected to different kinds of treatment. Still, further experiments *in vivo* are necessary to functionally validate the effects of HO-1 on apoptosis.

Furthermore, low HO-1 expression was associated with an increased risk of developing lymph node metastasis in OSCC (Tsuji *et al*, 1999) and tongue squamous cell carcinoma (Yanagawa *et al*, 2004). It could be associated with the lower expression of HO-1 in more undifferentiated cells (Yanagawa *et al*, 2004). By contrast, overexpression of HO-1 in pancreatic cancer cells increased the occurrence of metastasis, while inhibition of HO activity completely inhibited the occurrence of metastasis (Sunamura *et al*, 2003). Thus, the mechanisms of HO-1 in the metastatic potential of cancer cells is not recognized and may depend on the type of cancer or other, still not defined factors.

A growing body of evidence indicates that HO-1 activation may play a role in carcinogenesis and can potently influence the growth and metastasis of tumors, in which tumour microenvironment (Hanna *et al*, 2009), angiogenesis (Price and Thompson, 2002), etc. are implicated. The study of HO-1 in oral cancer has been relatively little and the related researches on HO-1 remain controversial or unconfirmed. The role of HO in the pathogenesis of oral cancer warrants further research.

HO-1 promoter polymorphism

HO-1 has been proposed to provide an important cellular response that protects cells against oxidative damage. However, humans differ quantitatively in their ability to mount a HO-1 response. The association between the HO-1 genotype and various oral diseases has been investigated. (GT)n repeats in the human HO-1 gene promoter are highly polymorphic, mainly because of variation in the number of (GT)n repeats. The longer (GT)n repeat exhibits lower HO-1 transcriptional activity (Chen *et al*, 2002) and the expression of HO-1 is regulated predominantly at the transcriptional level (Maines, 1988). Chang *et al* (2004) examine polymorphism in the HO-1 promoter in relation to the risk of OSCC in Asian male areca chewers. They find that the presence of long (GT)n repeats, defined as equal to or above 31 GT, is highly associated OSCC and the shorter (GT)n repeat, defined as equal to or below 25 GT, may have protective effects for OSCC. The same group recently published a paper demonstrating that OSCC with lymph node metastasis or advanced stage have significantly higher frequency of NFκB1 insertion and HO-1 long allelotypes (Lin *et al*, 2006). It is probable that the consensus sequences necessary for binding several regulatory factors including NFκB are present in the HO-1 gene promoter region (Muller *et al*, 1987).

Additionally, the (GT)n-repeat promoter polymorphism is investigated in 99 non-areca chewer OSCC patients that underwent complete surgical resection by Vashist *et al* (2008), who demonstrate that presence of short allele is associated with a lower tumor recurrence rate and better relapse-free survival in OSCC patients and propose that HO-1 promoter polymorphism might be considered as a potential prognostic marker in OSCC patients (Vashist *et al*, 2008).

In summary, in oral diseases, only in the field of OSCC, a potential impact of the (GT)n repeat polymorphism has been demonstrated, but other oral diseases may be worth examination with respect to a potential impact of HO-1 promoter variability.

Conclusions and future perspectives

It is almost certainly that HO-1 is up-regulated in diseases ranging from periodontal disease to oral cancer. HO-1 system may play an important role in oral diseases, pharmacologic modulation of which may represent an effective and cooperative strategy to

intervene. On the one hand, since HO system has shown antioxidant, anti-apoptotic and anti-inflammatory properties, up-regulating the system through HO-1 inducer such as hemin (iron protoporphyrin IX, FePP), which is successfully formulated as a powder for inhalation and the inhalation model allowed controlled HO-1 expression treating chronic inflammation in the lungs of mice (Zijlstra *et al*, 2007), can trigger HO-1 pathway of cellular defense in oral mucosa. Subsequently, the endogenous protective potential of the system may be achieved for preventing and treating inflammatory oral diseases. On the other, HO-1 is very often upregulated in oral cancers, which cytoprotective and antiapoptotic activities can improve survival of tumor cell and resistance to different types of therapies. Thus, Jozkowicz *et al* (2007) propose that HO-1 can be considered a 'friend' protecting healthy tissues from induction of some types of cancers. However, once the disease start to develop, HO-1 changes into a 'false friend,' as it will protect the tumor cells facilitate tumor progression. Therefore, down regulating the HO system by pharmacological or genetic means will be a new therapeutic approach in the managements of oral cancers. A comprehensive understanding of the underlying mechanisms for the observed effects of HO and its products will be necessary before their use can be evaluated in clinical applications for the prevention and/or treatment of human diseases such as oral diseases.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NO.: 30070952; 30472248) and Science and Technology Planning Project of Guangdong Province, China (NO.: 2006B35601007).

Author contributions

Wenguo Fan wrote the manuscript. Xiao Zhu, Dongpei Li, Shenli Fu, provided data and assisted with the drafting. Fang Huang and Hongwen He reviewed the manuscript.

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