Heme Oxygenase-1: Clinical Relevance in Ischemic Stroke

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Abstract: Stroke is the second-leading cause of death and a leading cause of serious long-term disability worldwide, with an increasing global burden due to the growing and aging population. However, strict eligibility criteria for current treatment opportunities make novel therapeutic approaches desirable. Oxidative stress plays a pivotal role during cerebral ischemia, eventually leading to neuronal injury and cell death. The significant correlation between redox imbalance and ischemic stroke has led to various treatment strategies targeting the endogenous antioxidant system in order to ameliorate the adverse prognosis in patients with cerebral infarction. One of the most extensively investigated cellular defense pathway in this regard is the Nrf2-heme oxygenase-1 (HO-1) axis. In this review, our aim is to focus on the potential clinical relevance of targeting the HO-1 pathway in ischemic stroke.

Keywords: Ischemic stroke, oxidative stress, heme oxygenase-1, cerebral ischemia, cerebral infarction, antioxidant system.

1. INTRODUCTION

Ischemic stroke is an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction [1]. Accounted for over 6 million deaths worldwide per year, stroke is the second-leading global cause of death and a leading cause of serious long-term disability [2]. Due to the growing and aging population, the global burden of cerebral infarction increases, in spite of a significant decline in age-standardized stroke rates [3, 4]. At the current state of the art, the only evidence-based acute treatment opportunities remain intravenous thrombolysis (within 4.5 hours after symptom onset) with alteplase and, in selected cases, endovascular intervention with mechanical thrombectomy [5]. However, due to the very narrow therapeutic time-window and various exclusion criteria, estimates of eligibility for systemic treatment within the population of ischemic stroke patients range only from 6% to 8% [6]. Furthermore, even if recanalization is achieved, the activation of a multi-step cascade, involving inflammatory immune responses, disruption of calcium homeostasis and enhanced production of Reactive Oxygen Species (ROS), can result in reperfusion injury [7]. Oxidative stress - with ROS causing damage to cellular components, including lipids, proteins and nucleic acids - plays a pivotal role during cerebral ischemia, eventually leading to neuronal injury and cell death [8-10]. The significant correlation between oxidative stress and ischemic stroke has led to various treatment strategies to ameliorate the adverse prognosis in patients.

Among transcription factors, nuclear factor erythroid 2-related factor 2 (Nrf2) plays a central role in cellular defence against oxidative stress [11]. Under conditions of redox imbalance, Nrf2 activates various detoxifying and antioxidant enzymes, such as NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferases (GSTs), heme oxygenase-1 (HO-1) and γ-glutamylcysteine synthetase (γ-GCS), by binding to antioxidant-response elements (AREs) and thereby inducing gene expression [12]. Among abovementioned phase 2 enzymes, HO-1 has been reported to have the most AREs on its promoter [13], making it a promising therapeutic target against brain injury in cerebral infarction. While HO-2 (expressed predominantly in the central nervous system) and the poorly characterized HO-3 are constitutive isoforms [14, 15], HO-1 - also referred to as heat shock protein 32 [16] - is the inducible type of heme oxygenase. It can be activated by various stimuli, including infections, heavy metals, ultraviolet irradiation, fever, inflammatory cytokines and oxidized Low-Density Lipoprotein (LDL) [17]. HO-1 catalyzes heme degradation, producing equimolar amounts of ferrous iron (Fe²⁺), Carbon Monoxide (CO) and biliverdin-IXa. Liberated free iron ultimately induces ferritin expression leading to Fe²⁺ sequestration and thereby limiting iron-mediated cell injury [18, 19], whereas biliverdin-IXa is rapidly converted to bilirubin-IXa by biliverdin reductase [20]. Among downstream products of HO-1, CO has emerged to have vasorelaxant, anti-inflammatory and anti-apoptotic properties [21], while biliverdin and bilirubin are known as potent endogenous antioxidant and anti-inflammatory molecules [20]. Furthermore, ferritin has also been demonstrated to exert anti-inflammatory effects [22]. The protective role of the Nrf2-HO-1 pathway in ischemic stroke is summarized in Fig. 1.

In this review, our aim is to focus on the potential clinical relevance of targeting the HO-1 pathway in ischemic stroke.

2. THE HO-1 PATHWAY AND ISCHEMIC STROKE: PRECLINICAL RESULTS

Among various animal stroke models, intra-arterial suture occlusion of the Middle Cerebral Artery (MCAO) has been used in more than 40% of the approximately 2,800 ischemic stroke experiments [23, 24]. The MCAO model is considered to be suitable for imitating human ischemic stroke with consecutive neuronal cell death, cerebral inflammation and blood-brain barrier damage [23, 24].

An early experiment using permanent MCAO in transgenic mice demonstrated that overexpression of HO-1 significantly reduces infarct volume [25]. On the other hand, HO-1 knockout mice showed significantly larger infarct size as compared to their wild-type counterparts [26].

In addition to genetically modified rodents, gene transfer vectors offer a further genetic strategy in preclinical experimental
stroke. In a rat MCAO model using stereotaxic instrumentation, treatment with an adenoviral vector overexpressing HO-1 resulted in decreased infarct volume and attenuation of neurologic deficits [27]. Because of the deleterious side effects of viral vectors, such as immunogenicity and oncogenicity [28], non-viral gene carriers have been investigated and were shown to induce site-specific HO-1 gene expression and therapeutic effect when applied as stereotaxic injection into the rat ischemic brain [29]. HO-1 gene transfer into injured carotid arteries of ApoE-null mice led to earlier thrombolysis with decreased plasminogen activator-1 expression [30].

Inducers of HO-1 have also demonstrated promising results in preclinical studies. Numerous natural compounds that activate the Nrf2-HO-1 pathway, such as sulforaphane [31], the standardized Ginkgo biloba extract EGb761 [26], curcumin [32], polyphenols [33, 34] and triterpenoids [35, 36] exert neuroprotective effects against stroke in animal models. Among pharmaceutical inducers of the Nrf2-HO-1 pathway, dimethyl fumarate (DMF) - approved for clinical use in the treatment of patients with relapsing forms of multiple sclerosis [37] - showed dose-dependent neuroprotection in a mouse MCAO model resulting in significant reduction of infarct volume, neurological deficits, brain edema and cell death [38]. In another study in mice, DMF was able to prevent blood-brain barrier hyperpermeability during ischemic stroke if given as pretreatment before ischemia-reperfusion injury [39]. Other drugs on the market with the ability to increase HO-1 expression include aspirin [40], statins [41], candesartan [42] and metformin [43]. However, regarding statins, two recent human studies did not confirm HO-1 induction in subjects treated with clinically relevant doses of simvastatin and atorvastatin [44, 45] which could at least partially be explained by the discrepancy in concentrations used in experimental situations and those detected in humans [46].

In addition to genetic and pharmacologic strategies in order to enhance HO-1 expression or activity, direct application or HO-1 endproducts offers the additional potential to harness the therapeutic potential of the HO-1 pathway. In a permanent MCAO model, mice exposed to low-concentration CO after permanent ischemia had significantly reduced infarct size, an effect which was abolished in Nrf2-knockout species suggesting that CO-exposure after stroke can provide protection by activating the Nrf2 pathway [47]. Of note is that delaying CO-exposure resulted in weaker protection suggesting a therapeutic time window. In another mouse MCAO model, Zeylanov et al. showed that inhalation of CO attenuated infarct volume significantly, limited brain edema formation and improved neurological deficit scores [48]. In a further study, intravenously applied PEGylated COHb in a transient focal cerebral ischemia model in rats resulted in reduced infarct volumes and better neurologic deficit scores [49]. Besides CO, biliverdin treatment has also been shown to ameliorate oxidative injury on neurons and decrease infarct size in rat MCAO models [50, 51].

A novel therapeutic approach to cerebral infarction is the use of stem cells. In a rat model of Hypoxic-Ischemic Brain Damage (HIBD), where placenta-derived mesenchymal stem cells (PD-MSCs) were injected with a stereotaxic apparatus, subjects receiving PD-MSCs showed significant improvement in HIBD along with a pronounced elevation in HO-1 and Nrf2 levels [52]. In an in vitro study, neural stem cells (NSCs) were pretreated with the polyphenol resveratrol prior to oxygen-glucose deprivation/reoxygenation. The authors found that resveratrol markedly increased NSC survival and proliferation, whilst upregulating expression levels of Nrf2 and HO-1 [53].

3. THE HO-1 PATHWAY IN HUMANS - CARDIOVASCULAR RISK FACTORS

3.1. HO-1 Gene Polymorphisms and Cardiovascular Risk

In humans, HO-1 expression shows a broad variability due to a highly polymorphic promoter, which has been correlated with cardiovascular risk factors. The most extensively studied variant is the length of the (GT)n-repeat region, which has been inversely related to HO-1 expression [54]. In a prospective, population-based survey,
3.2. Atherosclerosis

Oxidized low-density lipoproteins along with ROS play a central role in atherogenesis and can induce HO-1 [60, 61]. The role of HO-1 in the protection of the vascular wall from atherosclerosis was unraveled by the first reported HO-1 deficient patient, whose autopsy report revealed fatty streaks and fibrous plaques in the aorta at the age of six [62]. LDL isolated from the plasma of this child showed practically no oxidative resistance [63]. The presence of HO-1 was found in human atherosclerotic plaques with no HO-1 expression in normal arteries [64]. Also, expression increases with the severity of atherosclerosis [65]. The prooxidant environment in the advanced atheromatous lesion precipitates erythrocyte lysis and the oxidation of liberated hemoglobin to ferri- and ferrymeglobin, while the released heme and iron promote further oxidation of lipids [66]. These events amplify endothelial cell cytotoxicity of plaque components which is inhibited by HO-1. Induction of HO-1 was found to be a stabilizing factor of vulnerable plaques by reducing necrotic core size and intraplaque lipid accumulation, whereas increasing cap thickness and vascular smooth muscle cells [67]. In coronary arteries obtained from Japanese autopsy cases, the prevalence of HO-1 expression increased as the lesion type and grade of stenosis progressed and was significantly higher in diabetic patients [68]. A study examining carotid artery plaques removed during endarterectomy found a strong association between Helicobacter pylori infection and expression of HO-1, predominantly in specimens obtained from asymptomatic patients. The authors concluded that oxidative stress elicited by the Helicobacter pylori infection may have been inhibited by HO-1, resulting in the stabilization of the atherogenic process [69]. Among downstream products of HO-1, a reciprocal relationship between serum bilirubin levels and carotid atherosclerosis has already been reported in prior studies [70, 71]. Furthermore, in a meta-analysis of 11 studies, increased serum bilirubin levels were found to be a decreased risk for the development of atherosclerosis [72].

3.3. Hypertension

Data regarding the relationship between HO-1 expression and hypertension are based on animal experiments using spontaneously hypertensive rats. Using chronic angiotensin-II infusion in a hypertensive rat model, pressure overload upregulated HO-1 expression and activity in the aorta [73]. Furthermore, it has also been shown that transferring human HO-1 into spontaneously hypertensive rats resulted in attenuation of the development of hypertension attributed to the vasodilatory effects of CO [74]. In human studies, lung tissues of newborns suffering from congenital diaphragmatic hernia and pulmonary hypertension showed reduced expression of HO-1 [75]. The blood pressure lowering effect of a 3-month treatment with olmesartan in essential hypertension, was, in part, attributed to an increase in plasma HO-1 levels [76]. Among the catabolic metabolites of HO-1, concentrations of bilirubin have been shown to be significantly decreased in patients with untreated hypertension, whereas not differing between normotensive and treated hypertensive subjects [77]. Furthermore, in a 10-year health monitoring Korean study with normotensive subjects, serum bilirubin levels and the incidence of developing hypertension were found to be correlating negatively [78]. In women with gestational hypertension or pre-eclampsia, end-tidal breath CO levels were significantly lower than in healthy controls [79].

3.4. Diabetes Mellitus

HO-1 expression and diabetes have been shown to be reciprocally related in animal experiments, e.g., overexpressing HO-1 slowed the progression of diabetes in NOD mice or induction of HO-1 in Zucker diabetic rats improved insulin sensitivity [80, 81]. In humans, HO-1 expression was found to be reduced in peripheral blood mononuclear cells of diabetic patients [82], whereas high expression levels of HO-1 in early pregnancy has been associated with reduced risk of gestational diabetes [83]. Furthermore, elevated plasma HO-1 concentrations were affiliated with higher odds ratios for developing type II diabetes mellitus [84]. The HO-1 inducer Nrf2 has been shown to be lower in pre-diabetic and diabetic patients, suggesting that attenuated antioxidant mechanisms due to decreased Nrf2 might be involved in the development of diabetes [85]. Individuals with higher serum levels of bilirubin were associated with odds of having a lower incidence of diabetes mellitus [86]. In patients with Gilbert syndrome and diabetes, hyperbilirubinaemia is correlated with a lower prevalence of vascular complications compared with patients having diabetes alone [87]. Based on abovementioned results, atazanavir-induced experimental hyperbilirubinaemia in type II diabetic patients over a 3-day treatment in a double-blind, placebo-controlled study, was associated with a significant improvement of endothelial function [88].

3.5. Obesity

Increased expression of HO-1 was observed in adipocytes obtained from patients with morbid obesity [89, 90]. Furthermore, nonsmoking bariatric patients were shown to have increased carboxyhemoglobin concentrations, as an indicator of HO-1 upregulation [91]. Data regarding HO-1, obesity and insulin resistance are controversial with one study indicating that diminished upregulation of HO-1 in visceral adipose tissue correlated with waist-to-hip ratio and insulin resistance in humans [89], whereas other authors demonstrated increased HO-1 expression in insulin-resistant obese patients with increased waist-hip-ratio when compared to insulin-sensitive obese controls [92]. A reverse correlation between bilirubin concentrations and abdominal obesity has been found in two studies [93, 94]. Moreover, Andersson et al. presented a linear reciprocity between weight loss and increase in serum bilirubin concentrations [95].

3.6. Cigarette Smoking

As a further risk factor for cardiovascular disease, cigarette smoking has been shown to increase HO-1 expression [96]. The relationship between cardiovascular risk factors and HO-1 expression is summarized in Fig. 2.

4. THE HO-1 PATHWAY IN PATIENTS WITH ISCHEMIC STROKE

Current American acute ischemic stroke treatment guidelines do not recommend the use of any neuroprotective agents [5]. Nevertheless, edaravone (MCI-186), a free-radical scavenger implicated
to exert neuroprotection through the HO-1 pathway [97], is recommended by the Japanese guidelines for acute ischemic stroke within 24 h from symptom-onset [98].

A prospective cohort study in an Asian population investigated the association between HO-1 gene T(-413)A polymorphism and clinical prognosis in patients with atherosclerotic stroke, concluding that patients with at least one A allele had significantly better outcomes than patients with TT genotype, possibly due to the high expression level of HO-1 [99]. Two studies in ischemic stroke patients evaluated (GT)n-repeat-polymorphisms in the HO-1 promoter region. In the first one, authors found that carriers of short (<25) GT-repeats in the HO-1 gene promoter exhibited a reduced risk for stroke or TIA but only in the absence of elevated plasma lipids, implying that genetic polymorphisms have moderate effects compared to traditional risk factors like hyperlipidaemia [100]. The second study concluded that patients carrying longer (>26) GT-repeats may have greater susceptibility to develop ischemic stroke, but only in the presence of low HDL-C, implying that the insufficient antioxidant effect of low HDL-C levels may be compensated by shorter GT-repeats and thus, higher HO-1 induction [101]. It is of note, however, that the HO-1 inducer heme arginate was able to increase HO-1 in humans irrespective of the (GT)n phenotype [102]. A further study showed that stroke patients had higher serum levels of HO-1 compared to patients with transient ischemic attack (TIA), which was explained by the exposure to a high degree of oxidative stress caused by atherosclerosis, infection and hypertension leading to an upregulation of HO-1 [15].

Downstream metabolites of HO-1 may also exert protective functions in human ischemic stroke. The abovementioned study by Li et al. showed that stroke patients also had lower levels of total and direct bilirubin compared to patients with TIA [15]. A prospective Korean study suggested that elevated serum bilirubin might offer some protection against stroke risk in men [103]. In a large, community-based sample, however, higher exhaled CO was associated with a greater burden of subclinical cerebrovascular disease and with increased risk of stroke/TIA. It remains unknown, whether elevated endogenous CO concentration serves as a causative factor of cerebrovascular adverse events, or represents a result of oxidative stress and thus, upregulated HO-1 activity [104].

Alpha-lipoic acid, a thiol antioxidant has been shown to exert neuroprotective effects and promote recovery after ischemic stroke in animal studies by attenuating oxidative stress, partially mediated by the HO-1 pathway [105]. In a retrospective clinical study of patients with diabetes who were treated with thrombolysis due to acute ischemic stroke, among those who were using alpha-lipoic acid at the acute stage of stroke, favorable outcomes occurred at significantly higher rates both at 3 months and 1 year [106].

While neuroprotective agents are generally considered to be well-tolerated and safe, the phase III trial for the Nrf2/HO-1 inducer triterpenoid bardoxolone-methyl (BARD) designed for the treatment of patients with type II diabetes and stage 4 chronic kidney disease (BEACOM trial) has failed due to adverse cardiovascular events [107].

**CONCLUSION**

While the desire to find or develop effective neuroprotectants is high, to date, none of the numerous neuroprotective agents tested in preclinical research has yielded any positive outcomes in human studies [108, 109]. Main challenges to adapt experimental findings to human research include age-related issues, safety concerns, optimal dosage and delivery, and cell/tissue-specific expression. Based on abovementioned hurdles resulting in unsatisfactory translational power, the Stroke Therapy Academic Industry Roundtable (STAIR) has been established to improve the quality of preclinical stroke studies [110], thereby ultimately extending the currently narrow treatment palette of ischemic stroke and eventually improving the life quality of stroke patients.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.
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