

Heme oxygenase-1 improves the survival of ischemic skin flaps (Review)

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Abstract. Heat shock protein 32 (Hsp32), also known as heme oxygenase-1 (HO-1), is an enzyme that exists in micro-somes. HO-1 can be induced by a variety of stimuli, including heavy metals, heat shock, inflammatory stimuli, heme and its derivatives, stress, hypoxia, and biological hormones. HO-1 is the rate-limiting enzyme of heme catabolism, which splits heme into biliverdin, carbon monoxide (CO) and iron. The metabolites of HO-1 have anti-inflammatory and anti-oxidant effects, and provide protection to the cardiovascular system and transplanted organs. This review summarizes the biological characteristics of HO-1 and the functional significance of its products, and specifically elaborates on its protective effect on skin flaps. HO-1 improves the survival rate of ischemic skin flaps through anti-inflammatory, anti-oxidant and vasodilatory effects of enzymatic reaction products. In particular, this review focuses on the role of carbon monoxide (CO), one of the primary metabolites of HO-1, in flap survival and discusses the feasibility and existing challenges of HO-1 in flap surgery.

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1. Introduction

In the fields of plastic surgery, hand and foot surgery, and burn treatment, skin flaps are particularly important for the closure of tissue defects. However, the ability to attenuate or even abolish the necrosis that occurs on skin flaps, mainly due to ischemia, remains challenging. Necrosis of the skin flap is not only related to anatomical factors of the skin flap itself (1-3), but also to some high-risk factors of the patient, such as age and diabetes (4-7). For example, Las *et al* (8) reported that diabetes, excessive anesthesia time and postoperative wound infection are risk factors for the failure of free flaps. Bekara *et al* (5) showed that an age older than 60 years, or the presence of diabetes and arterial diseases are risk factors for complications of lower extremity pedicle perforator flaps. In addition, Sanati-Mehrziy *et al* (4) reported that smoking and operation time are risk factors for free flap necrosis and de Blacam *et al* (7) found that venous congestion and increased age are risk factors for pedicle flap failure. Current literature regarding the risk factors of skin flap necrosis mainly focus on free skin flaps and lower limb pedicled skin flaps (8-15), and treatment methods that have been proposed are based on various mechanisms such as surgical delay, chemical delay, extracorporeal shock wave therapy, local thermal pretreatment, percutaneous neuroelectric pretreatment, cold pretreatment, negative pressure suction, targeted gene therapy, and drug injection (16-25). Heat shock proteins (Hsps), which are highly evolutionarily conserved from prokaryotes to human (26), are molecular chaperons that exhibit a variety of biological activities including anti-oxidative, anti-apoptotic and anti-inflammatory effects (27). In recent years, several Hsps have been shown to prevent skin flap necrosis by reducing inflammation, oxidative stress, apoptosis and regulating platelet activity (28-32).

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Abbreviations: Hsp32, heat shock protein 32; HO-1, heme oxygenase-1; CO, carbon monoxide; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; ROS, reactive oxygen species; CORM, carbon monoxide-releasing molecules; BBB, blood-brain barrier; ICAM-1, intercellular adhesion molecule-1; NF-κB, nuclear factor κB; iNOS, inducible nitric oxide synthase; PMN, polymorphonuclear; TNF-α, tumor necrosis factor α; IL, interleukin; MIP, macrophage inflammatory protein; hBVR, human biliverdin reductase; ERK, extracellular signal regulated kinase; RONS, reactive oxygen and nitrogen species; eNOS, endothelial nitric oxide synthase; PKC, protein kinase C; MAPK, mitogen activated protein kinase; HIF-1α, hypoxia-inducible factor 1α; Nrf2, nuclear factor erythroid-2-related factor 2

Key words: heme oxygenase-1, skin flap, heat shock protein, carbon monoxide, preconditioning

Hsp32, a member of the Hsp family also known as heme oxygenase (HO)-1, is the most commonly studied molecule in the HO family of proteins. However, clinical data concerning HO-1 in flap surgery are scarce. This review summarizes the biological characteristics of HO-1 and the functional significance of its products. In particular, this review focuses on the role of carbon monoxide (CO), one of the primary metabolites of HO-1, in flap survival, elaborates on the protective effect of HO-1 on skin flaps (Table I), and discusses the feasibility and existing challenges of HO-1 in flap surgery.

2. Biological characteristics of HO-1

The human *HO-1* gene, located on chromosome 22q12, is approximately 1414 kb in length and contains four introns and five exons. The length of the (GT)_n microsatellite structure is unique to the promoter region of the human *HO-1* gene and affects its transcription. A longer (GT)_n leads to more GT dinucleotide sequence repeats and decreased *HO-1* gene transcription and expression levels (33).

The HO family consists of three members: HO-1, HO-2 and HO-3. HO-1 is the rate-limiting enzyme of heme metabolism (34), but the functions of HO-2 and HO-3 remain elusive. HO-1, with a molecular weight of 32 kDa, is a stress protein that is either not expressed or has exceptionally low expression under normal conditions. However, HO-1 can be induced by a variety of stimuli, including heavy metals, heat shock, inflammatory stimuli, heme and its derivatives, stress, hypoxia, and biological hormones (35-39). Increased HO-1 expression under stress reduces protein oxidation and lipid peroxidation, and attenuates cell and blood vessel damage, thus playing a protective role (40,41). For instance, Chen *et al* (40) reported that stress significantly increases HO-1 expression in the heart, which in turn provides cardioprotection. On the other hand, most metalloporphyrins, including tin protoporphyrin and zinc protoporphyrin, are inhibitors of HO-1 and compete with heme for the HO-1 binding site, thereby inhibiting the biological effects of HO-1 (42,43). The molecular weights of HO-2 and HO-3 are 36 kDa and 33 kDa, respectively (44). It is known that adrenal cortex hormones can induce the expression of HO-2, and it is therefore speculated that HO-2 can regulate functions within the nervous system. HO-3 has 90% primary structure homology with HO-2 but does not have any enzymatic activity. It is speculated that HO-3 regulates heme-dependent functions within cells (45).

3. HO-1-mediated enzymatic cascades and the functional significance of its products

HO-1 exists in microsomes and is the rate-limiting enzyme for heme catabolism, which splits heme into biliverdin, CO and iron. Biliverdin is further reduced to bilirubin under the action of biliverdin reductase, which is an effective anti-oxidant that protects cells from oxidative stress (46,47). Another metabolite, CO, acts on soluble guanylate cyclase (sGC) to increase the production of cyclic guanosine monophosphate (cGMP), which serves as a second messenger to regulate a wide spectrum of cellular events, including vasodilation (48,49) as well as anti-inflammatory and anti-apoptotic activities (50) (Fig. 1).

Heme. Heme is a pro-oxidant molecule that participates in the formation of oxidative free radicals leading to oxidative damage (51). In a model of heme overload using nude mice, excessive heme in the plasma promotes the production of reactive oxygen species (ROS) and reduces the effectiveness of nitric oxide (NO), thereby affecting the expansion of blood vessels (52). HO-1 degrades heme, and therefore has anti-oxidant activity. Balla *et al* (51) showed that the combination of heme and ferritin, together with HO-1, prevents oxidative stress. In another study performed in cultured alveolar epithelial cells, HO-1 overexpression was found to lead to a nearly 3-fold increase in ferritin, which was accompanied by a compensatory increase in transferrin receptors, subsequently altering the distribution of iron in the cells and protecting cells from iron toxicity induced by heme degradation (53).

CO anti-inflammatory activity. The anti-inflammatory activity of CO and CO-releasing molecules (CORM) has been well documented in various animal models. Increased HO-1 expression or CO prevents impairment of the blood-brain barrier (BBB), cerebral microvascular congestion and neuroinflammation (54). Mechanistically, this protection is achieved by the binding of CO to hemoglobin to prevent its oxidation and the production of free hemoglobin (54). In addition, a carrageenan-induced inflammation model showed that CO inhibits neutrophil migration and white blood cell adhesion (55). On the other hand, CORM-2 reduces the accumulation of inflammatory cells, the expression of intercellular adhesion molecule-1 (ICAM-1) and the activation of nuclear factor κ B (NF- κ B) in septic mice (56). *In vitro* experimental study confirmed that the mechanisms behind the anti-inflammatory effect of CORM-2 are related to the decreased expression of NF- κ B-dependent vascular endothelial cell adhesion molecules and secretion of inducible nitric oxide synthase (iNOS) (56). Using an animal model related to human sepsis (cecal ligation and puncture in rodents), CORM-2 or CORM-3 was found to reduce the migration of polymorphonuclear (PMN) leukocytes to purulent tissues, thereby attenuating inflammation and saving animals from succumbing to sepsis (57-59). In HO-1-deficient mice, treatment with CORM-2 was found to ameliorate the severity of sepsis associated with *Enterococcus faecalis* infection (60). CORM-3 was found to have a bactericidal effect on *Pseudomonas aeruginosa*, thereby reducing the mortality of mice with bacteremia (61). Additionally, CO exerts prominent anti-inflammatory actions in part by reducing the release of tumor necrosis factor α (TNF- α), interleukin (IL)-1, macrophage inflammatory protein (MIP)-1 and IL-6 from activated macrophages (62,63).

Protective effect of CO in cardiovascular diseases. The beneficial effects of CO on the cardiovascular system have been gradually discovered. Due to severe arterial thrombosis, HO-1-deficient mice died within four days following an aortic transplantation; however, treatment with CORM-2 was found to improve the survival rate of HO-1-deficient mice (64). The underlying protective mechanisms are related to the inhibition of platelet aggregation through the activation of guanylate cyclase (65). Endogenous

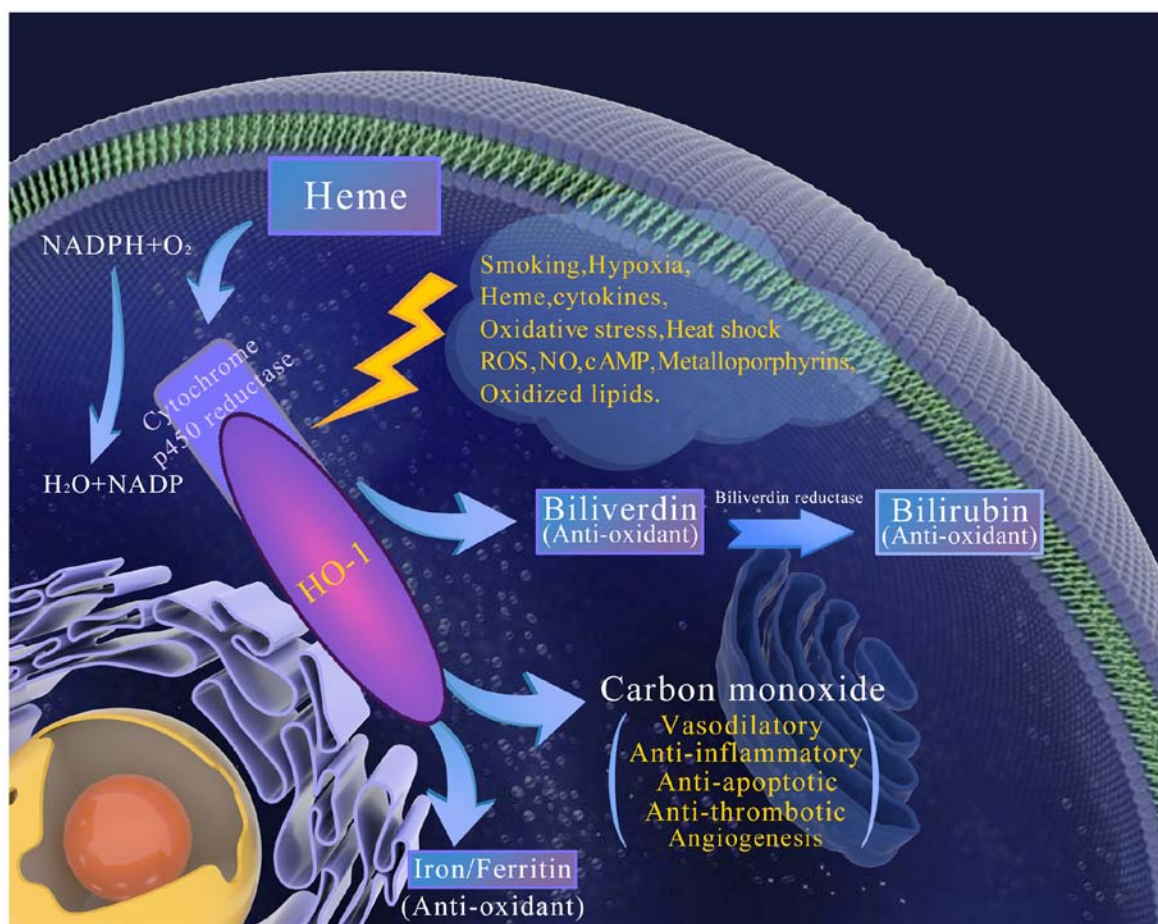


Figure 1. Biological activities of HO-1 and its byproducts. A variety of stimuli can produce HO-1, which in turn degrades the pro-oxidant heme, and produce effective anti-oxidant molecules, bile pigments and vasodilator carbon monoxide (CO) molecules. HO-1, heme oxygenase-1; ROS, reactive oxygen species.

CO binds to and activates sGC, subsequently increasing the concentration of cGMP and inducing a vasodilatory response (50,66). In a mouse model of myocardial infarction caused by coronary artery ligation, intravenous infusion of CORM-3 prior to reperfusion was found to reduce infarct size, fibrillation and tachycardia (67,68). The mechanisms underlying CORM-3-mediated cardiovascular protection involve potassium channels, since a small amount of CORM-3 is lost when mitochondrial ATP-dependent potassium channel inhibitors are used (67,68). Another study also demonstrated that HO-1 promotes angiogenesis through CO (69), and improves the survival of tissues surrounding the blood supply by inhibiting apoptosis of endothelial cells (70).

Preservation of transplanted organs. Many scholars have reported that CO plays an important role in organ transplantation and preservation. CO in a gaseous form or as a CORM can be used as a preservation adjuvant in organ preservation solution. Using a model of allogeneic heart transplantation in rodents, continuous inhalation of CO or CORM can prolong the survival time of the transplanted heart (71,72). The cardioprotective effect of CO gas is achieved by inhibiting platelet aggregation and endothelial cell apoptosis, as well as producing endogenous vasoconstrictors, thereby improving the microcirculation of the

graft (71,72). Compared with the untreated control group, rabbit kidneys washed with CORM-3 or CORM-1 and then stored in a cold solution for 24 h showed higher rates of perfusion flow, glomerular filtration, and sodium and glucose reabsorption (73). The above-mentioned mechanisms of renal protection are thought to be related to CO-mediated expansion of blood vessels, inhibition of cell apoptosis and promotion of angiogenesis (73).

Bilirubin and biliverdin. As previously mentioned, HO-1 generates biliverdin, which is then converted to bilirubin. Both bilirubin and biliverdin are effective oxidative inhibitors (74). Human biliverdin reductase (hBVR), a Ser/Thr/Tyr kinase, is activated by insulin and free radicals (75). hBVR is central to the activation as well as the nuclear import and export of extracellular signal-regulated kinase (ERK)1/2 (76). Insulin increases BVR tyrosine phosphorylation and increases glucose uptake when BVR is knocked down by small interfering RNA, suggesting an important function of BVR in insulin signaling (77). HO-1-derived bilirubin is an efficient scavenger of reactive oxygen and nitrogen species (RONS) (78). In macrophages, biliverdin induces the phosphorylation of endothelial NO synthase (eNOS) at Ser1177, which generates eNOS. It is well recognized that NO plays a crucial role in regulating a wide spectrum of vascular functions, including vasorelaxation, inhibition of leukocyte-endothelial adhesion,

vascular smooth muscle cell migration and proliferation, and platelet aggregation (79).

In summary, each product generated by HO-1 plays an important role in protecting cells and tissues through a variety of mechanisms.

4. Protective effects of HO-1 on skin flaps

Anti-inflammatory effect. The survival rate of skin flaps is related to ischemia-reperfusion injury and surgical trauma. Ischemia-reperfusion injury, if not treated in a timely and appropriate manner, may cause unfavorable outcomes for patients. Both surgical trauma and ischemia-reperfusion may initiate inflammatory responses, causing white blood cells to roll and adhere to the capillary vein (80-82). In the early reperfusion phase, the accumulation of activated leukocytes and reactive oxygen metabolites aggravates the inflammatory response and ultimately impairs endothelial integrity (83). The initiation of the stress response leads to increased HO-1, which significantly reduces the adhesion of white blood cells to the endothelial surface and lessens the impairment of the integrity of venous endothelium (84). Rücker *et al* (84) studied whether stress conditioning-induced HO-1 could prevent an inflammatory response in transferred osteomyocutaneous flaps. In all tissues analyzed, control flaps presented with significant leukocyte adherence in postcapillary venules, increased expression of intercellular adhesion molecule-1 (ICAM-1), and disruption of endothelial integrity. In contrast, stress conditioning induced considerable HO-1 expression, which coincided with a significant reduction of leukocyte adherence, ICAM-1 expression, and endothelial hyperpermeability. Correspondingly, the inhibition of HO-1 by tin protoporphyrin IX completely abolished the stress conditioning-induced amelioration of the inflammatory response (84). Hence, the protective effect elicited by stress conditioning is mainly mediated by the induction of HO-1, which reduces oxygen free radicals, ICAM-1, adherence of white blood cells to the endothelial surface, endothelial permeability and macromolecular extravasation, ultimately reducing the overall inflammatory response.

Anti-oxidative effect. Rats undergoing flap reconstruction surgery experience lipid peroxidation and vascular damage, which is related to ischemia-reperfusion injury (85). Ischemia-reperfusion injury involves a complex oxidation process, which is closely related to flap survival (86). Oxidative stress induces an excessive activation of inflammatory processes, which increases ROS. ROS are molecules known to cause tissue damage through multiple mechanisms, including altering the structure and chemistry of proteins, lipids, and nucleic acids. Hence, reducing the release of ROS or removing excessive ROS protects tissues from ischemia-reperfusion injury (87). Lin *et al* (88) reported that CORM-2 induces HO-1 expression, thereby reducing protein kinase C (PKC)/amino acid-rich tyrosine kinase 2 (Pyk2)-dependent production of ROS. Lin *et al* (28) showed that ginkgolide B reduced skin flap necrosis and improved the survival of island perforator flaps by inhibiting endoplasmic reticulum stress and oxidative stress. In this study, they further showed that ginkgolide B activated

nuclear factor erythroid 2-related factor 2 (Nrf2) signaling. Following its activation, Nrf2 is transported to the nucleus, where it binds to antioxidant response elements located in the *cis*-regulatory sequences of antioxidant-related enzymes and proteins and increases their expression (89).

Anti-apoptosis, vasodilation and angiogenesis. Brouard *et al* (90) studied the anti-apoptotic potential of HO-1 in cultured endothelial cells and found that CO, the product of heme digested by HO-1, activates the p38 mitogen-activated protein kinase (MAPK) pathway to inhibit endothelial cell apoptosis. But the HO-1 agonist heme arginate (HA) failed to improve the survival rate of ischemic flaps (91). *In vitro* studies have shown that HA is cytotoxic to keratinocytes (91). Brouard *et al* (90) also found that CO causes vasodilation, which is part of the mechanism by which HO-1 protects skin flaps from chronic ischemia-induced injury (90). Chronic ischemia was found to increase the expression of HO-1, especially on the first and third days post-operation. Increased HO-1 expression in turn induces arteriole expansion and promotes perfusion, which can maintain sufficient capillary perfusion density in the flap and reduce flap necrosis (92). The inhibition of endogenous HO-1 by tin protoporphyrin IX was found to completely eliminate arteriole expansion and hyperperfusion, resulting in a significant decrease in functional capillary density, a significant increase in cell apoptosis, and skin flap necrosis, but aging can reverse this protective effect (92-94). Consistent with the above findings, Kubulus *et al* (95) reported that delay-associated tissue protection can be obtained by HO-1-mediated attenuation of microcirculatory dysfunction. Increased HO-1 activity protects tissues by biliverdin-associated anti-oxidative actions and/or CO-mediated vasodilation. This is supported by findings that show that trolox treatment after selective blockade of HO-1 reduced necrosis only until day three after flap creation and did not affect the manifestation of microcirculatory disorders. These observations suggested that: i) oxidative stress contributes to the initial development of flap necrosis; ii) anti-oxidative treatment counteracts oxidative stress-induced cell damage; iii) the initial oxidative stress does not define the degree or severity of final flap necrosis; and iv) HO-1 prevents flap necrosis induced by chronic ischemic conditioning (delay) primarily through its vasodilatory action and by improving microvascular perfusion (95). Kubulus *et al* (23) further showed that cooling induced a marked expression of HO-1 without induction of the Hsp70 protein, which was accompanied by significant improvement in microvascular perfusion. Thus, HO-1 also plays a role in the cooling-mediated amelioration of microcirculation, which results in a significant reduction in final flap necrosis. In another independent study, Sun *et al* (96) showed that preconditioning with isoflurane promotes hypoxic stabilization and induces the accumulation of hypoxia-inducible factor 1 α (HIF-1 α), while HIF-1 α mediates transcriptional activation of the HO-1 gene in response to hypoxia to induce angiogenesis.

However, research has also shown that HO-1 may have dual functions. For example, HO-1 has a dual role in cancer cells. The levels of cellular iron and ROS are the determining factors for the role of HO-1, in which excessive cellular iron and ROS tend to push HO-1 from a protective

Table I. Summary of the literature regarding Hsp32 application in the field of flap surgery.

Authors (ref.)	Year	Research subject	Skin flap model	Stimulus	Major findings (flap necrosis: Control vs. stimulus)
Rücker <i>et al</i> (36)	2001	Rat	Osteomyocutaneous flaps of left hindlimb	Local heat-shock	Stress initiated by local heat shock improved nutrient perfusion of the musculocutaneous flap through HO-1-elicited capillary dilation.
Rücker <i>et al</i> (84)	2001	Rat	Osteomyocutaneous flaps of left hindlimb	Local heat-shock	Local heat shock-induced stress increased HO-1 expression in musculocutaneous flaps, which was related to a significant decrease in leukocyte adhesion, ICAM-1 expression and high endothelial permeability. Hsp32 inhibitors completely abolished the anti-inflammatory effect of HO-1. These observations suggest that local heat shock-initiated stress can reduce the inflammatory response of the musculocutaneous flap.
Harder <i>et al</i> (93)	2004	Pig	A random-pattern skin flap was collected on both sides of the buttocks	Local heat preconditioning	Local heat preconditioning significantly reduced ischemic flap necrosis by decreasing complications of ischemia-related wound healing (40 ± 8 vs. $7 \pm 14\%$, $P < 0.01$).
Kubulus <i>et al</i> (95)	2004	Mouse	Axial-pattern skin flap of the ear	Surgical delay	Overall protection against flap necrosis by chronic ischemic conditioning (delay) was mediated by HO-1, not through an anti-oxidative function but rather through a vasodilatory action, which prevented microvascular perfusion failure (41 ± 3 vs. $16 \pm 3\%$, $P < 0.05$).
Harder <i>et al</i> (34)	2005	Mouse	A random-pattern myocutaneous flap was collected on the back	Local heat preconditioning	Local heat preconditioning of myocutaneous tissue markedly increased flap survival by maintaining adequate nutrient perfusion rather than inducing ischemic tolerance (53 ± 5 vs. $4 \pm 1\%$, $P < 0.001$).
Chang <i>et al</i> (37)	2005	Human	Gingival fibroblasts and tissues were obtained from cigarette smokers and non-smokers	Nicotine	The expression of HO-1 in gingival tissues of smokers was significantly upregulated; nicotine and other components may be a reason for the increased expression of HO-1. Regulation of nicotine-induced HO-1 expression was heavily dependent on the intracellular concentration of GSH.
Kubulus <i>et al</i> (23)	2005	Mouse	Axial-pattern skin flap of the ear	Cooling-induced preconditioning	Cold pretreatment induced significant expression of HO-1, but not Hsp70 protein. HO-1 induced capillary dilation, thereby improving microvascular perfusion.
Harder <i>et al</i> (94)	2007	Mouse	Axial-pattern skin flap of the ear	Aging	The increased susceptibility to ischemic necrosis during aging is more likely due to a loss of vascular reactivity to endogenous HO-1, resulting in a lack of adequate adaptation to chronically ischemic conditions [$49 \pm 8\%$ ($P < 0.05$) and $42 \pm 8\%$ ($P < 0.05$) in aging and adult patients, compared with $31 \pm 6\%$ in young patients].

Table I. Continued.

Authors (ref.)	Year	Research subject	Skin flap model	Stimulus	Major findings (flap necrosis: Control vs. stimulus)
Contaldo <i>et al</i> (38)	2007	Pig	A random-pattern skin flap was raised on both sides of the buttocks	MPL and local hyperthermia	iNOS and HO-1 were upregulated after MPL and local hyperthermia ($P<0.05$), but only local hyperthermia significantly reduced the necrosis rate of skin flaps [44% in local hyperthermia, compared with 29% in control ($P<0.05$)].
Harder <i>et al</i> (92)	2008	Mouse	Random flap on the back	Chronic ischemia	Chronic ischemia-induced endogenous HO-1 protected ischemic tissues through the vasodilatory action of HO-1-associated CO release (73 ± 5 vs. $51\pm5\%$, $P<0.001$).
Schürmann <i>et al</i> (39)	2009	Mouse	Caudally pedicled skin flap on the back	Surgery of a caudally-based skin flap	Skin flap epithelial keratinocytes presented a prominent inflammatory response upon surgery, which was not amplified but rather controlled by invading HO-1 expressing-macrophages in the surviving flap tissues.
Sun <i>et al</i> (96)	2013	Human cells and rat	Random-pattern skin flaps on back	ISO	ISO preconditioning improved survival of skin flaps by upregulating the expression of HIF-1 α , HO-1 and VEGF (51.5 ± 5.6 vs. $43.3\pm6.7\%$, $P<0.05$).
Edmunds <i>et al</i> (91)	2014	Rat and human epidermal keratinocytes (HEKs)	A transverse rectus abdominomyocutaneous flap	HA	Compared with the control group, the HO-1 agonist HA failed to improve the survival rate of ischemic flaps (Compared with the control group, skin flap necrosis was more than 30% higher in HO-1 agonist HA group; $P=0.002$). <i>In vitro</i> studies have shown that HA is cytotoxic to keratinocytes.
Han <i>et al</i> (86)	2015	Rat	Abdominal rectangular random flap	ADSCs	Adjuvant treatment with ADSCs significantly increased the survival of a skin flap in the venous ischemia-reperfusion condition. This effect was achieved through the suppression of the inflammatory response and induction of the antioxidative response (68.7 ± 11.9 vs. $48.4\pm13.6\%$, $P<0.05$).
Lin <i>et al</i> (28)	2019	Rat	Multiterritory perforator flap on the back	GB	GB reduced oxidative stress through the activation of Nrf2/HO-1 signaling and enhancement of antioxidant activity (37.03 ± 6.50 vs. $9.17\pm1.93\%$, $P<0.01$).

Hsp, heat shock protein; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule-1; GSH, glutathione; iNOS, inducible nitric oxide synthase; MPL, ministration of monophosphoryl lipid A; HIF-1 α , hypoxia-inducible factor 1 α ; VEGF, vascular endothelial growth factor; HA, hemearginate; ISO, isoflurane; ADSCs, adipose-derived stem cells; GB, ginkgolide B; Nrf2, nuclear factor erythroid-2-related factor 2.

role to that of a perpetrator. In general, a moderate level of HO-1 activation exerts a cytoprotective effect, while the over-activation of HO-1 becomes cytotoxic due to

the excessive increase of labile Fe²⁺ behind the buffering capacity of ferritin (97). Similarly, HO-1 also has a dual role in nerve cells. Nrf2-dependent activation of HO-1 is

generally linked to protective effects in neurons and glial cells, while Nrf2-independent activation of HO-1, which often involves AP-1 or NF- κ B, seems to exert neurotoxic effects. Indeed, HO-1 expression is associated with neuronal damage and neurodegeneration, especially in Alzheimer's and Parkinson's disease (98).

The application of CO, the catalytic product of HO-1, within a clinical setting also has the following issues. For example, the safety constraints of CO require clinical dosing to maintain levels of carboxyhemoglobin (CO-Hb) that are under 14%, which is in stark contrast to the preclinical frontrunner studies with protocols that had sustained CO-Hb levels above 20% (99). Also, the pharmacokinetics of CO inhalation protocols varied significantly by species. Rodent models achieve therapeutic levels quickly, whereas the time to steady state in humans is roughly three times longer based on respiratory rate and cardiac output (100).

Similarly, the application of HO-1 or its metabolites to the field of flap surgery will also face the above-mentioned issues. Instead, perhaps it would be possible to: i) use HO-1 inhibitors (such as tin protoporphyrin) to repress its overexpression; ii) use an occasional monitoring system to achieve feedback to control the level of CO in the body; or iii) search for molecules that promote the release of CO or solutions that can dissolve CO, and apply them locally to reduce systemic adverse reactions.

5. Perspectives

HO-1 was first described in the late 1960s and its functions have been gradually characterized over the following decades, innovative observations have been made over the past 20 years uncovering the cellular cytoprotective capability of HO-1 in a clinical setting. While studies have defined to some degree the tissue protective roles of each of the by-products generated by HO-1 (including CO) and attempted to harness HO-1 to improve CO-delivery for clinical applications, there are still significant limitations that prevent its use at the bed side (99). Previous studies have shown the clinical relevance of HO-1, such as in ischemic stroke (101), skin health (102) and cardiovascular syndromes and co-morbidities (103). However, there are few studies on the application of HO-1 in the field of human flap surgery. How to direct the clinical application of HO-1 and its related products in the field of flap surgery in order to obtain more appreciable outcomes will be the focus of future research.

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Authors' contributions

YZ drafted the manuscript. ZL assisted with the literature search. MY assisted with drafting and revising the manuscript. XG conceived and designed the review. YZ and XG are responsible for confirming the authenticity of the raw data. All authors read and approved the final manuscript and agree to be accountable for the accuracy and referencing of the information included in the review.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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