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Research paper

Localized delivery of carbon monoxide

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ABSTRACT

The heme oxygenase (HO)/carbon monoxide (CO) system is a physiological feedback loop orchestrating various cell-protective effects in response to cellular stress. The therapeutic use of CO is impeded by safety challenges as a result of high CO-Hemoglobin formation following non-targeted, systemic administration jeopardizing successful CO therapies as of this biological barrier. Another caveat is the use of CO-Releasing Molecules containing toxicologically critical transition metals. An emerging number of local delivery approaches addressing these issues have recently been introduced and provide exciting new starting points for translating the fascinating preclinical potential of CO into a clinical setting. This review will discuss these approaches and link to future delivery strategies aiming at establishing CO as a safe and effective medication of tomorrow.

1. Introduction

The heme oxygenase (HO)/carbon monoxide (CO) system is an important cellular feedback-loop towards oxidative and inflammatory insults in man (Fig. 1) [3]. The central building block of this system is the HO-1, an inducible enzyme catalyzing heme degradation into CO, biliverdin, and Fe^{2+}. Detailed reports on cell-protective, anti-oxidative, anti-apoptotic, and anti-inflammatory effects of the HO-1 and its effectors (including CO) have previously been outlined [3]. Polymorphisms impairing upregulation of this enzyme have been linked to various pathophysiological conditions including coronary artery disease [4], diabetes [5], or the outcome of organ transplantations [6]. The disease modifying impact of CO was demonstrated in disease models mainly by systemic application either directly applying CO mostly through inhalation or CO-Releasing Molecules (CORM) [3]. Both modalities face substantial challenges driving the need for novel approaches as discussed below. Inhaled CO leads to one principal challenge of this (and other systemic) administration routes as of the rather exhaustive binding of CO to hemoglobin (Hb), e.g. following diffusion through the alveolar membrane when pulmonary administered. Therefore, systemic trafficking of pulmonary delivered CO is mainly in a tightly bound form as the carboxyhemoglobin (CO-Hb) complex and very little 'free' CO is available to act pharmacologically (Fig. 2A). Noteworthy, CO also tightly binds to a variety of other heme-containing proteins including myoglobin [7], neuroglobin [8], and cytochrome *C* oxidase [9]. For simplification and comparability among various preclinical and clinical reports this review will focus on CO-Hb among these CO binding moieties. The extraordinary stability of CO-Hb requires quite high CO-Hb plasma concentrations such that therapeutically relevant levels of free CO are achieved – critically narrowing the therapeutic window [10]. This conundrum has to be solved for any systemic CO delivery attempt, as very high, perhaps toxic CO-Hb levels have to be condoned in order to achieve therapeutically relevant levels of free CO in the target tissues (Fig. 2A) [11]. This disadvantageous prerequisite is also true for the delivery of CO using systemically administered CORMs. On top, these CORMs aggravate the development risk in that they further hold potentially toxic transition metals required for binding CO (Fig. 2A) [3,12].

Therefore, and in spite of the exciting therapeutic potential of CO, current systemic application strategies resulted in an uphill battle against both, the CO-Hb to free CO ratio and potential systemic safety concerns associated with the molecular scaffolds most CORMs deploy. Consequently, novel developments point to local delivery and targeting approaches addressing these concerns. These include CORMs that decarbonylate in diseased hepatic tissue (thereby, the CO is carried into the liver before it releases from the CORM – therapeutically relevant free CO is increased while CO-Hb percentages can be kept low) as well as Drug Delivery Systems (DDS) that locally control CO release in the gastrointestinal (GI) tract (thereby further reducing unfavorable CO-Hb binding while maximizing exposure of the target tissue to free CO) [13,14]. This review will start off past and current CO delivery concepts and therapeutic evidence, define desirable pharmaceutical specifications turning CO into a druggable molecule and finally link to

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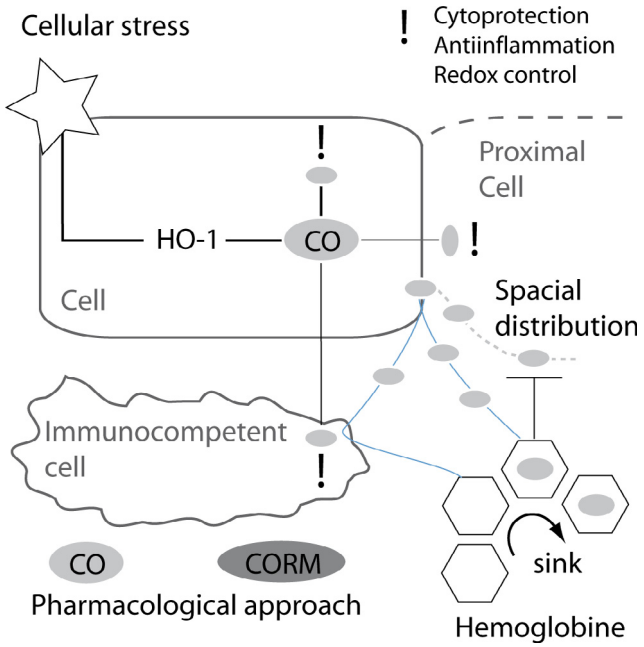

Fig. 1. The heme oxygenase (HO)/carbon monoxide (CO) system as an important cellular feedback-loop towards local oxidative and inflammatory insults. Following cellular stress the HO-1 is induced catalyzing heme degradation into CO, biliverdin, and Fe^{2+} (latter two are not shown for simplification) thereby orchestrating various cellular response mechanisms and triggering auto- as well as paracrine effects. Counterbalancing this effect by hampering the unhindered spatial distribution of CO is hemoglobin constantly removing CO with the blood flow. Approaches for mimicking this feedback loop include the inhalation of CO as well as the application of Carbon Monoxide Releasing Molecules (CORMs).

future delivery strategies aiming at translating CO into effective medication of tomorrow.

Nature's equivalent for the therapeutic approaches discussed here within is HO-1 being central to the local feedback loop of the HO/CO system (Fig. 1). Technologies designed for mimicking this loop, therefore, preferably simulate (i) signaling and (ii) the special spatiotemporal characteristics of this system (*vide infra*). Resembling the function as a cellular sentinel, the enzyme is induced by an array of diverse imbalances including inflammation, radiation, and hypoxia [15]. Multiple transcriptional factors including activator protein 1 (AP-1), NF-κB, nuclear factor like 2 (NRF-2), cAMP response element-binding protein (CREB), and enhancer box (E-Box) induce HO-1 expression and are themselves controlled through a multitude of pathways including extracellular-signal regulated kinases (ERK), p38 mitogen-activated protein kinases (p38 MAPK), as well as c-Jun N-terminal kinases (JNK) activation [15]. Thereby, HO-1 orchestrates a network of para- and autocrine events providing a protective response (Fig. 1). Autocrine effects are mainly attributed to the high diffusivity and stability of CO, a special characteristic that differentiates CO from other signaling molecules [16]. Hemoglobin is tightly controlling this spatial distribution by instantaneously scavenging CO following passage to the capillary networks (Fig. 1) [17].

The mobility of CO as well as its ability to induce HO-1 has been discussed as important features for effective exogenous application of CO and CORMs [16,18]. Likewise, genuine HO-1 inducers including cobalt protoporphyrin and hemin have been used as almost equivalent lab stage alternative [3,19]. Induction is also observed in response to various other compounds including drugs like curcumin [20], acetylsalicylic acid [21], resveratrol [22], lansoprazole [23,24], and 5-aminosalic acid [25]. The lack of selectivity of this mode of action leading into numerous downstream effects, however, challenged the development of HO-1 inducers for therapeutic purposes [26,27]. Until today, induction of HO-1 in man was solely demonstrated for hemin [28], which failed in a trial profiling for gastroparesis both in prolonged HO-1 induction (>7 days) and ameliorating the disease activity [29] contrasting previous preclinical trials [30,31]. Furthermore, hemin is a neurotoxic [32], fairly unselective molecule that besides HO-1 induction represses δ-aminolevulinic acid synthase, the rate-limiting enzyme for the synthesis of tetrapyrrols [15,33]. This feature is clinically relevant for the treatment of acute porphyria – a rare disease characterized by porphyrin accumulation – for which hemin infusions are used [34]. Therefore, novel effectors of the HO-1 pathways are required and CO is a promising option discussed here within.

2. Systemic application of CO

2.1. Transferability of preclinical results

The systemic application of CO by inhalation demonstrated efficacy in numerous preclinical models [3] contrasting clinical trials: Promising preclinical evidence on endotoxemia [35,36] was not confirmed in a clinical trial exposing healthy volunteers to 500 ppm CO for one hour resulting in 7% CO-Hb [37]. Likewise, a safety and tolerability study using inhaled CO in kidney transplant patients motivated by promising preclinical results [38–40] was withdrawn for unknown reason [41]. Other trials focused on local exposure of the lungs through inhaled CO for the treatment of pulmonary diseases. One study on chronic obstructive pulmonary disease (COPD) demonstrated a trend to disease modification following inhalation of 100 ppm CO (resulting CO-Hb was 2.6% of total Hb) [42]. Three further clinical trials assessing the potential in arterial hypertension, acute respiratory distress syndrome, and idiopathic pulmonary fibrosis following CO inhalation are ongoing [43–45]. Systemic CO levels can be compared among preclinical and clinical studies using the amount of CO-Hb formation among species for relative comparison [46]. Due to toxicity challenges (*vide infra*) clinically applied CO was limited to doses equivalent to CO-Hb levels of <10%. In contrast the majority of preclinical trials have been conducted with 250–500 ppm CO gas [3] resulting in CO-Hb levels above 20% (Fig. 2B) [47–51]. A solid data package linking CO-Hb concentrations to anti-inflammatory and cell protective effects is not available to date (*vide infra*). The fundamental mechanism of this lack of transferability from animal model systems to humans is not well understood, however, dissimilarities in quantities applied as well as pharmacokinetic differences among species in the specific context of therapeutic gas delivery had been suggested as major translational hurdles [1,13,52]. To this end, particularly important issues are differences in absorption and elimination kinetics among species, with 4 times slower CO-Hb formation patterns and 8 times slower reconstitution of hemoglobin in man as compared to rodents, respectively (Fig. 2B) [53–55]. Consequently, 15 h exposure is required in humans to reach steady state whereas only 4 h are required in rodents (modeled for 100 ppm CO enriched breathable air in Fig. 2B). Exposing patients for 15 h to CO enriched breathable air is likely not viable. However, loading these patients with initially high doses of CO (e.g. >1000 ppm) and in an effort to reduce inhalation times to some hours is challenged by potential toxicities. To illustrate this further: therapeutically relevant CO levels in patients (>10% CO-Hb, *vide supra*, Fig. 2B) are achieved following inhalation of either 100 ppm for 15 h (steady state) or 1000 ppm CO for 30 min (Coburn-Foster-Kane (CFK) equation, see Supplementary Table 1) [1,54]. Proceeding inhalation of 1000 ppm for further 30 min would translate into levels of >25% CO-Hb, quantities which potentially trigger severe toxicity and illustrating the delicate handling of inhalation when using highly CO enriched breathing air (Fig. 2B)