Cardiac function dependence on carbon monoxide

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Abstract

Nitric oxide, studied to evaluate its role in cardiovascular physiology, has cardioprotective and therapeutic effects in cellular signaling, mitochondrial function, and in regulating inflammatory processes. Heme oxygenase (major role in catabolism of heme into biliverdin, carbon monoxide (CO), and iron) has similar effects as well. CO has been suggested as the molecule that is responsible for many of the above mentioned cytoprotective and therapeutic pathways as CO is a signaling molecule in the control of physiological functions. This is counterintuitive as toxic effects are related to its binding to hemoglobin. However, CO is normally produced in the body. Experimental evidence indicates that this toxic gas, CO, exerts cytoprotective properties related to cellular stress including the heart and is being assessed for its cytoprotective and cytotherapeutic properties. While survival of adult cardiomyocytes depends on oxidative phosphorylation (survival and resulting cardiac function is impaired by mitochondrial damage), mitochondrial biogenesis is modified by the heme oxygenase-1/CO system and can result in promotion of mitochondrial biogenesis by associating mitochondrial redox status to the redox-active transcription factors. It has been suggested that the heme oxygenase-1/CO system is important in differentiation of embryonic stem cells and maturation of cardiomyocytes which is thought to mitigate progression of degenerative cardiovascular diseases. Effects on other cardiac cells are being studied. Acute exposure to air pollution (and, therefore, CO) is associated with cardiovascular mortality, myocardial infarction, and heart failure, but changes in the endogenous heme oxygenase-1 system (and, thereby, CO) positively affect cardiovascular health. We will review the effect of CO on heart health and function in this article.

Key words: carbon monoxide; cardiac function; cardiac physiology; cardiomyocytes; cell physiology; gasotransmitters; heart; mitochondria

INTRODUCTION

Nitric oxide, studied to evaluate its role in cardiovascular physiology, has cardioprotective and therapeutic effects in cellular signaling, mitochondrial function, and in regulating inflammatory processes. Heme oxygenase (HO; major role in catabolism of heme into biliverdin, carbon monoxide (CO), and iron) has similar effects as well. CO has been suggested as the molecule that is responsible for many of the above mentioned cytoprotective and therapeutic pathways as CO is a signaling molecule in the control of physiological functions. This is counterintuitive as toxic effects are related to its binding to hemoglobin. However, CO is normally produced in the body. Experimental evidence indicates that this toxic gas, CO, exerts cytoprotective properties related to cellular stress including the heart and is being assessed for its cytoprotective and cytotherapeutic properties. While survival of adult cardiomyocytes depends on oxidative phosphorylation (survival and resulting cardiac function is impaired by mitochondrial damage), mitochondrial biogenesis is modified by the HO-1/CO system and can result in promotion of mitochondrial biogenesis by associating mitochondrial redox status to the redox-active transcription factors. It has been suggested that the HO-1/CO system is important in differentiation of embryonic stem cells and maturation of cardiomyocytes which is thought to mitigate progression of degenerative cardiovascular diseases. Effects on other cardiac cells are being studied. Acute exposure to air pollution (and, therefore, CO) is associated with cardiovascular mortality, myocardial infarction, and heart failure, but changes in the endogenous HO-1 system (and, thereby, CO) positively affect cardiovascular health. We will review the effect of CO on heart health and function in this article. MEDLINE®, the Cochrane Central Register of Controlled Trials, and clinicaltrials.gov were searched for carbon monoxide, cardiac function and gasotransmitters, cardiac function and CO, mitochondria and CO, CO and cell physiology, gasotransmitters, carbon monoxide and cardiomyocytes, CO and the heart, CO and cardiac physiology.

PRODUCTION/REGULATION OF CARBON MONOXIDE

Cytotoxic effects are seen when heme results in formation of oxygen free radicals and lipid peroxidation, but its degradation products (CO, biliverdin, bilirubin) mediate cytoprotection.[1] Synthesis of CO *in vivo* is normal and associated with heme catabolism requiring NADPH and O_2 as cofactors. The gasotransmitter binds to ferrous heme – structures regulating the function of cells are altered and heme proteins and/or enzymes affected.[2] Heme metabolism accounts for at least 86% of CO in the body. Iron-ascorbate catalyzed lipid peroxidation of microsomal lipids and phospholipids, reduction of cytochrome b5, auto- and enzymatic oxidation of phenols, and photo-oxidation of organic compounds are heme-independent sources of the gasotransmitter. The gas membrane permeability is high and may result in changes in the autocrine, paracrine, and endocrine systems. Formation is tightly controlled, requires enzymes, and occurs in the body. Effects are observed at varying concentrations and second messengers may be necessary. There are both molecular and cellular targets. CO, nitric oxide (NO), and hydrogen sulfide (H_2S) transgress cells and tissues rapidly and react with protein amino acids, metal groups, and reactive

chemical species. These major gasotransmitters (CO, NO, and H_2S and, possibly, ammonia and methane[3]) can impact each other as well as normal and abnormal biochemical, molecular, and biological functions.[4] While the gasotransmitters affect many common molecular targets, biochemical activities are through different mechanisms. Each gasotransmitter can affect the production of another gasotransmitter. Their function in physiological processes depends on the local environment, scavenging systems, effective gas concentration, and physio-chemical properties of the gas.[5] Endogenous formation of CO usually requires the presence of HOs.

Arterial blood CO is a reflection of degradation of heme.[6] Synthesized through multiple steps which are catalyzed by eight enzymes, heme is degraded by HO which results in the breakdown of heme to CO, ferrous ions and biliverdin. HOs are products of the expression of different genes with differences in the number of isoforms in different species.[7] HO-1 (inducible form, heat shock protein 32, identified in 1974), the major physiologically relevant mechanism of heme catabolism in mammals, is a 32-kDa protein and is cytoprotective against oxidative stress. Primarily localized in endoplasmic reticulum, HO-1 also is found in mitochondria, peroxisomes of parenchymal and nonparenchymal liver cells, cytoplasm, and nuclear matrix. HO-2 (constitutive form, identified in 1986) is anchored to the endoplasmic reticulum by a hydrophobic sequence of amino acids at the carboxyl terminus of the protein and/or may be anchored to plasma membranes directly or indirectly through big-conductance KCa channels, has a mass of 36 kDa, has high concentrations in the brain, and depolarization of neurons results in increased activity with an associated increase in CO. HO-3 (constitutive form, identified in 1997) is 33 kDa and has a homology to HO-2 (shares about 90% amino acids) but activity is low and, in cells, is a heme-binding protein, regulates production of free radicals, and is thought to act as an oxygen sensor.[8-10] Little is known about this latter isoform.

There is variability in expression and regulation of the HOs and, therefore, the amounts of CO production. Interaction between isoforms HO-1 and HO-2 may limit heme oxyenase activity in some tissues where the two co-exist.[11] The fluctuation in CO levels may result in changes in cellular and extracellular interactions and affect normal biology and physiology. While CO has a strong affinity for iron in hemoglobin (forming carboxyhemoglobin), it interacts with other Fe-containing molecules as well. Selectivity between NO, oxygen, and CO determines the physiological function of most heme proteins.[12] Excess CO is thought to be exhaled through the lungs.

The major endogenous source of CO production, heme degradation, does not happen when CO is present. Heme is a cofactor in oxygen transport proteins and enzymes involved in critical cellular processes and CO, a product of heme-protein turnover, has physiological effects which are dependent on interactions with complex heme moieties of the cellular hemo-proteins. While endogenous levels of CO are low, molecular and cellular effects are profound and important to normal physiology, lack of pathology, and repair mechanisms. Ubiquitous in nature, CO preferentially reacts with ferrous (Fe^{2+}) heme-dependent proteins and other transition metals in

a specific redox state. Presence or absence of target proteins affects the specificity of CO-mediated effects.[13] Heme based sensor proteins, regulators of cellular responses to changes in CO levels, act as signal transducers, couple a regulatory heme binding site to a functional signal-transmitter site, and regulate gas-generation and gas-reception mechanisms.[14,15] Crucial for signal transduction, heme-protein sensor selectivity is specific for NO, oxygen, or CO. More than 50 sensor proteins exhibiting four different types of heme-binding motifs (CO-sensing transcription factor A, heme-No-binding domains, heme-binding Per-Arnt-Sim (PAS), and globin-coupled sensor) have been identified.[16] Concentration, spatial localization, and temporal regulation determine CO effect. CO can be continuously produced by HO in the presence of oxygen and NADPH in small amounts - function in physiological processes depends on the amount of gas produced and delivered to a given system. Production depends on cell free hemoglobin which is oxidized by reactive oxygen species (ROS) to form met-hemoglobin that dissociates to hemin, the ferric form of the heme prosthetic group, and apo-globin. Hemin induces HO-1 – hemin is transported into cells and HO in the cells catalyzes degradation to the aforementioned components.[17] Level of HO-2 is fairly constant – heme catabolism is regulated by the inducible or repressible HO-1 (depends on cell types and/or microenvironments).[18]

HO-1, a mixed function oxidase, can be activated and confer cellular protection - cytoprotective effects are related to end-products.[19] Increased heme levels, depletion of intracellular glutathione, ultraviolet light, heat shock, various metals, environmental chemicals, hypoxia, hyperoxia, hydrogen peroxide, and NO induce HO-1. Presence of the stannous ion as opposed to the stannic ion increases the induction of HO-1 as well. The mitogen-activated protein kinase pathway was linked extracellular events to stress-mediated induction of HO-1. Expression is regulated by "leucine zipper" transcription factors and can function as homo-dimeric transcriptional complexes or as hetero-dimeric transcriptional complexes. Biliverdin reductase is a regulator of HO-1 expression as well.[20-23]

HO-2 also oxidizes heme to CO and biliverdin, is constitutively expressed in all cells with the promoter responding to glucocorticoids, is a substrate for biliverdin reductase kinase activity, is an oxygen sensor, binds heme to molecules not involved in the catalytic function of the oxygenase, regulates normal cell function, and is important in cellular homeostasis.[24-27] HO-2 is mainly located in microsomal fractions with HO-1 seen in the microsomal and cytosol fraction.[28] HO-2 activity depends on phosphorylation which is dependent on different protein kinases.[29] Expression of HO-2 is not strictly constitutive.[30,31] Activity is substrate dependent – those factors increasing heme availability also increase production of CO. HO-2 can also be activated through the thiol/disulfide redox molecular switch.[32]

CARBON MONOXIDE AND CARDIAC PHYSIOLOGY

While CO affects multiple cell types and physiological functions, there are overlapping biochemical activities of CO, NO, and H_2S that can impact normal and abnormal biofunctions. **Table 1** shows known functions of CO in the heart. This list is

Table 1: Different types of functions of carbon monoxide in the heart

Function
Bioenergetics involving mitochondria
Modulation of mitochondrial biogenesis
Embryonic stem cell differentiation
Cardiomyocyte maturation
Affects phenotype changes affecting apoptosis, proliferation, and inflammation
Target soluble guanylyl cyclase, nitric oxide synthase, NADPH oxidase, complex IV of mitochondrial electron transport chain, and mitogen-activated protein kinase pathway
Production of mitochondrial reactive oxygen species
Mitochondrial uncoupling factor
Limits hydrogen peroxide production
Limits mitochondrial membrane permeabilization
Increases ability of mitochondria to take up calcium
Increases tricarobxylic acid cycle rate
Increases oxidative phosphorylation
Increases mitochondrial biogenesis
Increases adenosine triphosphate production
Limits the activity of T-type and L-type calcium channels thereby preventing excitotoxicity-induced cell death
Modulates cell proliferation
Affects electrophysiological activity
Release of calcium from sarcoplasmic reticulum
Relaxation of blood vessels

expanding. Similar to cells outside of the heart, cellular signaling by CO regulates cellular activities at the molecular/cellular/ whole organ levels, is necessary for survival and growth of the organism, is required to assess the cellular environment, and is needed for intra- and intercellular communication. Known as a cellular asphyxiate, CO (the diatomic oxide of carbon) binding to hemoglobin diminishes the unloading of oxygen in all tissues. A Janus-molecule, CO is toxic at higher concentrations, but beneficial at lower – toxic properties are related to dosing. Increasingly, CO is considered important for the physiological regulation of organs and for restoring normalcy in disease.

A signal transmitter, endogenous CO is a stable non-radical and forms complexes with most known hemoproteins at the heme iron center to include NADPH:oxidase, myoglobin, cytochrome C oxidase, inducible NO synthase, cytochrome p-450, soluble guanylate cyclase, and the heme-HO complex.[13,33-35] Breakdown is by oxidation of CO to CO_2 which occurs in the mitochondria.[33] Substrate availability, pH, presence of regulatory proteins/cofactors, and oxygen levels affect intra and extracellular environment and, therefore, CO formation. Whether CO acts at the site where it is generated or affects other systems distant from its production is not fully understood. While cell stressors may result in cell death, cell proliferation, and neoplasia leading to cellular damage and, possibly, organ failure, endogenous CO can affect the response of the cell to stressors and result in a cytoprotective phenotypic change affecting apoptosis, proliferation, and inflammation.[36] Normal cell processes (including oxygen sensing, adenosine triphosphate (ATP) synthesis, apoptosis, proliferation,

cytoprotection, and cellular respiration) are affected and the respiratory complexes of the mitochondria are an important target for CO as well as NO and H_2S.[37] Established cellular targets of CO include soluble guanylyl cyclase, NO synthase, NADPH oxidase, complex IV of the mitochondrial electron transport chain, and the mitogen-activated protein kinase pathway. Deleterious and beneficial effects have been seen. The heart and the brain which normally consume oxygen are the most sensitive to CO-induced damage. CO protects against ischemia/reperfusion injury and dilates coronary blood vessels. Mitochondria are important to this process.

Mitochondria (essential for development and function of the myocardium) which are generators of ROS (cause oxidative damage), regulate physiological processes, and are a source of ATP energy, derive most of its energy from mitochondrial oxidative phosphorylation. Mitochondria provide large amounts of ATP needed by the heart, occupy a large volume of cardiomyocytes and half the volume of myofibrils, and are needed for cardiac development and function. They affect cardiomyocyte differentiation and survival, maturate cells and organelles (develop structurally, become functionally specialized in the heart, and increase mass,), modulate intracellular Ca^{2+} homeostasis and production of intermediary metabolites, are dependent on nutrient and oxygen supply, and undergo metabolic adaptation determined by the environment. The latter is disrupted in cardiovascular disease and results in a change of mitochondrial function which results in abnormalities in the respiratory chain and ATP synthesis, increased oxidative stress, and loss of structural integrity of the organelle.[38-41] Mitochondrial quality control through regulation of mitochondrial biogenesis and mitophagy are affected by gasotransmitters. Counter-inflammatory cytokine induction, interleukin-1 receptor antagonist, and suppression of cytokine synthesis 3 depend on mitochondrial biogenesis.[42,43] A cellular target for CO, mitochondrial biochemical pathways are critical to normal cardiac cellular physiology, contractility, and cardiac recovery - mitochondrial dysfunction and cardiomyocyte death are related to oxidative phosphorylation and energy efficiency. Endogenous CO results in the production of mitochondrial ROS (signaling molecules for CO-induced biochemical pathways which can result in cytoprotection) which promotes anti-inflammatory responses, stimulates mitochondria biogenesis and protects against cell death. HO-1 induction results when there is an increase in mitochondrial ROS.[44,45] CO is an anti-oxidant and is a mitochondrial uncoupling factor, limits hydrogen peroxide production, and may be the result of ROS generation at low levels. Mitochondrial membrane permeabilization (inhibiting release of pro-apoptotic factors into the cytosol) is limited by CO. This is associated with disruption of intracellular homeostasis and point of no return in the control of cell death. Mitochondrial uptake of Ca^{2+} is increased by CO as is tricarboxylic acid cycle rate, oxidative phosphorylation and mitochondrial biogenesis, and ATP production. CO also limits the activity of T-type and L-type Ca^{2+} channels thereby preventing excitotoxicity-induced cell death and modulates cell proliferation.[46]

Inherent to mitochondrial function and modulated by CO, ion channels (members of the P2X receptors, tandem P domain

K⁺ channels, epithelial Na⁺ channel, calcium-activated K⁺ (BKCa), and the voltage-activated K⁺ (Kv), and Ca²⁺ channels), regulate normal physiology and modulate ion fluxes across membranes (both plasma and organelle membranes.) Molecular mechanisms by which this is done are unclear – different mechanisms have been postulated. The most widely studied ion channel target, the BKCa channel (large-conductance Ca²⁺-activated potassium channels, regulate physiological functions tissues, composed of pore-forming α subunits and accessory β subunits), is probably activated by direct and indirect mechanisms.[47] It is unclear as to whether CO acts as a Ca²⁺ substitute in this channel, but CO increases BKCa channel activity. Jaggar et al.[48,49] found that CO regulates these channels by binding to reduced heme and CO sensitivity is independent of redox status. BKCa channel openers may have a protective effect on the cardiovascular system, vascular resistance, and functioning coronary flow. The mitochondrial large-conductance calcium-activated potassium channel (mitoBKCa) activity (has a central role in protecting the heart from ischemia, pharmacological activation affects the generation of ROS and mitochondrial Ca²⁺ which prevents cell death probably by interfering with the uncontrolled opening of the mitochondrial transition pore) is inhibited by heme alone but stimulated in the presence of heme and CO. Interaction with the channel has been suggested at non-heme sites.[50] CO also stimulates the soluble guanylyl cyclase/cyclic guanosinc monophosphate (cGMP)/protein kinase G pathway leading to channel phosphorylation and activation in the mitoBKCa.[33] Binding to to a high-affinity, channel-associated heme moiety on the α-subunit, to extracellular histidines, and CO interaction with the aspartate and histidine residues in the regulator of K⁺ conductance 1 have been suggested as mechanisms of action.[48,49,51,52] Phosphorylation of BKCa by protein kinase G at specific serines increases the probability of BKCa having an open conformation.[37] Intracellular concentration of cGMP is dependent on guanylate cyclase and cyclic nucleotide degrading enzymes (phosphodiesterases). Membrane bound guanylate cyclase and the soluble forms are activated through different mechanisms – natriuretic peptides for the membrane guanylate cyclase and NO and CO for the cytosolic guanylate cyclase. Intracellular cGMP signaling pathways result from its direct effect on neurotransmission, calcium homeostasis, platelet aggregation, heartbeat, bone remodeling, lipid metabolism, and activity of cation channels, activity of G protein kinases, phosphodiesterases, cyclic nucleotide dependent cation channels, Wnt proteins and sex hormones, and regulation of phototransduction (eye).[48,53-57] Jaggar et al.[48] determined that CO binds to endogenous channel-bound heme (altering the interaction between heme and the heme-binding domain) resulting in activation of BKCa channels. A required prosthetic group in many electron transfer proteins and redox enzymes, heme affinity for the heme binding domain of the BK channel (as well as CO and HO-2) regulates activity of the BK channel in response to intracellular hypoxic/normoxic changes, responds quickly and reversibly to changes in the redox state of the cell, and may depend on a thiol/disulfide switch in the heme binding domain.[56] The mechanisms that allow CO to regulate other ion channels may be direct or indirect, relate to the particular ion

channel, and depend on cellular redox state – CO selectively augments P2X2 receptors, CO sensitivity is conferred by a small number of residues in BKCa²⁺ and L-type Ca²⁺ channels, an inhibitory action of CO L-type Ca²⁺ channels may result in cardioprotective effects of HO-1, CO potentiation of L-type Ca²⁺ channel activity increases HIF-1α-independent vascular endothelial growth factor expression, etc.[56,58,59] Kv1.5 is inhibited by CO and involve multiple signaling pathways.[60] These known ion channel targets of CO (high-conductance Ca²⁺-sensitive K⁺ channel (BKCa) via augmentation by direct binding or binding via closely associated heme group, tandem P domain K⁺ channel by augmentation via protein kinase G activation at low concentrations and inhibition at higher concentrations, voltage-gate K⁺ channel (Kv2.1) by inhibition in part via increased ROS but also requiring protein kinase G activation, cardiac L-type Ca²⁺ channel (α1C subunit) by inhibition via increased production of ROS from mitochondria, intestinal smooth muscle L-type Ca²⁺ channel by augmentation via increased NO and cGMP with additional involvement of protein kinase A but not protein kinase G, epithelial Na⁺ channel by inhibition not via cGMP and augmentation, and ATP-gated P2X receptors by augmentation of P2X2 not via soluble guanylate cyclase and inhibition of P2X2/3 and P2X4 receptors with no effect on P2X3) result in physiological and pathological changes and are being evaluated as targets for therapeutic CO intervention.[61]

Heterocellular communications (signaling between cells) are facilitated by biochemical molecular modifications and affected by CO as well. Complexity increases as organ systems and organ interdependence develop. The multiple cell types within an organ system are different in morphology, metabolic function, biochemical and molecular reactions, and cellular physiological responses. The gasotransmitters serve important functions in regulating physiologic responses and effects of each gasotransmitter are different – activity, longevity, and potency vary between the gases and even for a single gas under different conditions. There are overlapping functions and formation of each gas is affected by environment. While CO, NO, and H₂S can interact with heme, molecular target interactions and metabolic products are important to stability of health and response in disease. Interconnectivity with each other complements individual functions. Intracellularly, CO, NO, and H₂S can be oxidized to form dinitrogen trioxide, nitrogen dioxide, nitrite, peroxynitrite, HNO, carbon dioxide, sulfenic acid, sulfinic acid, sulfonic acid, sulfite, and sulfate. Sulfenic acid can be reduced to free thiol by Trx or oxidized to sulfinic acid or sulfonic acid. CO binds to heme groups and blunts oxygen consumption by inhibiting mitochondrial COX activity. Cellular redox potential and membrane potentials are affected and intracellular organelles are targets of CO. Important in health, therapeutic implications are driving biomedical research in development of CO as a drug for diseases affecting a wide range of organ systems dependent on heterocellular communication. Complex interactions and different reaction products impact the final result of gasotransmitter therapy. Endogenous CO affects the various interactions in different ways depending on the need of the system. An improved understanding of these dynamic interactions within the car-

diovascular system is prerequisite to exogenous use of CO as a therapy in cardiovascular disease.[4,62-67]

CO and NO have similar affects – both act as messengers and signaling molecules. A more stable molecule than NO and H_2S, CO, a signaling molecule in the cardiovascular system, may exert effects for longer time periods and distances when compared with NO and H_2S.[10,68,69] Known effects of CO on the cardiovascular system include modulation of autonomic nervous system input to both the primary pacemaker and the working myocardium, vasodilation, and changes in heart rate and strength of contractions. Production of cardiovascular CO under basal conditions is continuous and, with upregulation of HOs, production should increase. Hemoproteins are targets of diffusing CO and NO synthases, heme-containing transcription factors, soluble guanylyl cyclase, and oxidases are some of the systems that have been identified as important targets in the heart. The upregulation of HO-1 seen with myocardial ischemia-reperfusion, heart preservation and transplantation, pulmonary hypertension and right heart failure, post-ischemia inflammation, and vascular dysfunction suggests that CO is necessary for cardiac health, its related physiology, and also has an antihypertensive effect.[70-74]

Electrical function of the heart is affected by CO and is determined by movement of ions across specific channels in cardiomyocytes with interactions of membrane channels working as part of complex biomolecular networks. A single ion channel protein may encounter and interact with hundreds of proteins during its existence and plasticity of these protein complexes are important in determining cardiomyocyte excitability, excitation-contraction coupling, and intercellular communication. Ion channel macromolecular complexes have several components which may include proteins, nucleic acids, carbohydrates and lipids and may perform various cellular tasks including metabolism, cell signaling, gene expression, trafficking, cell cycle regulation, and formation of subcellular structures. Electrophysiological activity in the heart is affected by CO either through direct actions or its effects on intracellular signaling pathways. Changes in NO through targeted nitrosylation of ion channels, ion transporters, or directly related signaling molecules are initiated by CO levels. NO/S-nitrosylation have been shown to increase the K^+ current that generates the resting potential in the heart, reduces the Kv1.5 repolarizing current in mammalian atria, can affect Na^+/K^+ ATPase turnover in the hypoxic heart, alters release of Ca^{2+} from the sarcoplasmic reticulum (SR) by targeting the ryanodine receptor complex in heart and skeletal membranes, and can increase the slowly inactivating or late Na^+ current (INa-L) in heart preparations.[75-79] CO affects changes in cardiac electrical activity, contractions, and cardiac arrhythmias through augmentation of slow inactivation of the cardiac Na^+ current – NO is a second messenger of this effect.[80,81] Mathematical simulations of CO effects on human ventricle potential show that CO acting through an intracellular NO-mediated signaling cascade can reduce peak INa, shifts the inactivation curve for the large transient component of INa in a hyperpolarizing direction, increases the slowly inactivating component INa-L, decreases the action potential rate-of-rise and increases action potential duration, changes ICa-L (which can produce early after-depolarization generation – NO driven S-nitrosylation can modify ICa-L), and results in an increase in SR Ca^{2+} which causes spontaneous and intermittent release of SR Ca^{2+} (results in inward current via the sarcolemmal Na^+/Ca^{2+} exchanger pathway – ICa-L and, possibly, INa-L may be enhanced and net inward current at the plateau level of the ventricular action potential is affected).[81-86]

Essential to normal cardiac function, intracellular calcium is critical to cardiac electrical activity and mechanical shortening of cardiomyocytes though a complex interaction of regulatory and structural contractile proteins. Movement of calcium into and out of cardiomyocytes and cycling of calcium between the cytosol and SR are needed for coordinated myocardial contraction. Changes in cytosolic Ca^{2+} are a result of the interplay of Ca^{2+} entry into the cell (largely via Cav1.2 channels), Ca^{2+} release from the SR, and cytosolic Ca^{2+} removal processes that extrude Ca^{2+} from the cell or return it back to the SR. Cardiac excitation-Ca^{2+} release coupling is dependent on sarcolemmal voltage-gated L-type Ca^{2+} channels and intracellular ryanodine receptors which communicate through the Ca^{2+}-induced Ca^{2+} release mechanism – Ca^{2+} stores regulate ryanodine receptor Ca^{2+} release channels via luminal and cytosolic Ca^{2+} sites.[87-90] Mitochondria and other organelles contribute to the calcium pool as well. CO increases the ability of mitochondria to take up Ca^{2+} – small amounts of Ca^{2+} increase mitochondrial metabolism.[46] SR-mitochondrial communication in cardiomyocytes (functional structure of T-tubules, junctional SR, and mitochondria) ensures efficiency of the excitation-contraction-bioenergetics coupling. Dependent on Ca^{2+} transfer from SR to mitochondria, uptake stimulates the formation of ATP needed to meet the energy demands of the heart. The process depends on Ca^{2+} transporting protein properties and spatial distribution.[91-94] The role of CO in this important process needs further study.

Relaxation of blood vessels, a component important to myocardial function, is through vasodilation and inhibition of the proliferation of vascular smooth muscle cells. A key event in atherogenesis, prominent in cardiovascular disease, is accumulation of vascular smooth muscle cells. This response to inflammatory stimuli commonly occurs in vessels with turbulent blood flow with subsequent hemolysis and free heme accumulation. Excess free heme catalyzes the formation of ROS – results in endothelial cell dysfunction and is associated with migration and proliferation of vascular smooth muscle cells – which activates redox-sensitive proliferation-related signaling routes and induces HO-1 expression. Attenuation of overall production of ROS is through the ability of HO to degrade heme and to produce CO, biliverdin/bilirubin, and release free iron. CO, a potent endogenous antioxidant, activates soluble guanylate cyclase, elevates cGMP in target tissues (dilates blood vessels), directly activates potassium channels in vascular smooth muscle cells, inhibits platelet aggregation and proliferation of vascular smooth muscle cells, inhibits apoptosis, and stimulates angiogenesis. Signaling pathways that involve NO/guanylyl cyclase, K^+ channels, ROS, and mitogen-activated protein kinases are modulated.[95-98] Arterial and venous blood vessels also express HO-2 which may regulate CO production and modulate vascular tone.[99] Targeting

of HO to affect the development of atherosclerosis, therefore, has therapeutic implications for diseases of the cardiovascular system and has been pharmacologically upregulated.

Also integral to cardiac function, cardiomyocytes have been said to have limited regenerative capacity to restore damaged tissue. However, the Bergmann group[100] predicted an approximate annual turnover rate of cardiomyoctes of about 1% at the age of 25 years and 0.45% by the age of 75 years. With injury, adult mammals do not regenerate the majority of the lost cardiomyocytes – necrotic tissue is replaced with scar tissue. This compromises contractility and may result in heart failure and death – reduction of cardiomyocyte loss and/or replacement of lost cardiomyocytes with newly generated counterparts is, therefore, important to restoring cardiac function. Heart regeneration models have shown that mechanisms include waves of inflammation, matrix deposition and remodeling, and cardiomyocyte proliferation is common.[101] Endogenous regeneration of the heart by activation of resident cardiac stem cells that can give rise to new cardiomyocytes after injury and reactivation of pre-existing cardiomyocytes (through proliferation, dedifferentiation, and redifferentiation) for expansion after injury have been suggested pathways to produce new cardiomyocytes.[100,102,103] Multipotent cardiac stem/progenitor cells that reside in the heart have been shown to differentiate into cardiomyocytes, smooth muscle cells, and vascular endothelial cells. Various cardiac stem/progenitor cells have been described, the most commonly described being c-kitpos Lineageneg cardiac stem cells, Sca-1+ cardiomyocyte progenitor cells, slide population cells, and cardiosphere-derived cells.[104] In a rat model of myocardial infarction, CO increased accumulation of c-kit+ stem/progenitor cells into the infarct area and induced formation of new coronary arteries by promoting differentiation of c-kit cells into vascular smooth muscle cells, increased proliferation of cardiomyocytes in the infarct border area, and had increased expression of hypoxia-inducible factor-1.[105] HO-1/CO induction may lead to mitochondrial ROS generation and upregulation of proteins required for mitochondrial DNA replication before stem cell differentiation. An RNA binding protein, CUG-BP Elav-like family 1, is higher in patients with hypertrophic cardiomyopathy, is a regulator of cardiomyocyte gene expression, and has lower HO-1 levels – a negative correlation with CUG-BP Elav-like family 1 and HO-1 (and CO).[106] The role of CO in cardiomyocyte regeneration requires further study.

Molecular cardiology has defined molecular pathways and genes involved in some cardiovascular diseases. Cardiovascular gene therapy is being evaluated as another therapeutic modality for inherited and acquired cardiovascular diseases. Gene therapy and gene transfer are powerful tools for studying the role of HO-1/HO-2 in the treatment of cardiovascular diseases.[107,108] Induction of HO-1 has been suggested as a mode of modifying the repairing abilities of stem cells in the heart. Functional recovery of infarcted heart has been observed with HO-1 transfection.[109,110] With myocardial infarction, HO-1 gene transfer has been correlated with an increase in neovascularization (vascular endothelial growth factor and stromal cell-derived factor-1 higher) and an increase in c-kit stem cells in the injured cardiac tissue.[111,112] Transference of

HO-1 and/or increasing its endogenous expression has been suggested as interventions for cardiac tissue repair. It is not clear if CO production as a result of increased HO-1 directly impacts repair of cardiac damage, but recent studies suggest that CO is critical to CO-mediated mitochondria biogenesis which is necessary for differentiation of embryonic stem cells into cardiomyocytes.[113,114] Administration of HO-1 in rats before ischemia/reperfusion injury reduces tissue damage suggesting HO-1 with a resultant increase in CO is a therapeutic strategy in cardiovascular disease.[105,107,115-119] The role of HO-1/CO pathway in generation and repair and/or regeneration of cardiac tissue requires further study.

CARBON MONOXIDE AND CARDIAC CELL BIOCHEMISTRY

Profiling of biomolecular markers of the heart in health and disease applies genomics, epigenetic, transcriptomic, proteomic, and metabolomics tools. Molecular causes of cardiac dysfunction are still largely unknown, but are expected to be the result of alterations in gene and protein expressions.[120-122] The proteome describes the total collection of proteins of an organism that are produced as a result of genome translation and post-translational modifications and is constantly changing because of physiological conditions and environmental challenges. Proteomics is the large-scale characterization of the entire protein complement of a biological system, is dependent on differences in proteome expression and configurations, and compares the proteome between health and disease to identify those differences in protein expression which allows the molecular phenotyping of a disease providing insight into the implicated molecular pathways. While genomics deals with the discovery and noting of all the sequences in the entire genome of a particular organism, proteomics focuses on the characterization of all proteins and their interactions within a biological system rather than on study of the structure and function of a single protein. The goal of cardiac proteomics is to understand how the structure and function of proteins allow them to do what they do, what they interact with, and how they contribute to cardiac processes (i.e., mitochondrial protein interactomes in the heart have been identified).[123] The role of CO in this process will help refine our understanding of normal and abnormal physiology and define therapeutic opportunities. For example, the contractile apparatus of the heart, dominated by a small number of proteins, differ between regions and cell types – human heart with four major cell types (cardiac fibroblasts, cardiomyocytes, smooth muscle cells, endothelial cells) – and is a prerequisite for cardiac physiology. Effects of CO on this dynamic process continue to be studied and its therapeutic roles determined. Nag and colleagues[124] showed that while cardiac muscle cells in culture maintain contractile activity and reduced cellular growth with exposure of CO in a concentration-dependent manner without the presence of oxygen, growth rate and contractility of cardiac myocytes with an oxygen tension maintained at 20% with 20% CO were higher than those of all other combinations. Even with high concentrations of CO, beating persists – necessary energy is available and cytochrome oxidase of heart muscle is able to oxidize CO. However, myofibrils of the control cells and those exposed to a mixture of 20% oxygen and 20% CO were

abundant and highly differentiated. Cardiac non-muscle cells were more affected by CO exposure than were the muscle cells – the latter are dependent on oxidative metabolism for cell division and subsequent survival. Mitochondria of degenerating or dead cells were extensively disorganized and disintegrated.[124] In another study, down regulation of cardiac contractile proteins with resultant myocardial depression during sepsis was mitigated by CO.[125] Using proteomics, Bauer and colleagues identified the structural protein vimentin as an additional vascular endothelial growth factor-HO-1 target in vascular endothelial growth factor-mediated angiogenesis.[126] Jin et al.[127] used proteomics to determine that HO-1/EBP interaction is protective in attenuating dysfunction of oxidative stress and cardiac systolic function induced by cholesterol stimulation. Heart maps detailing anatomy and cell types to determine abnormal biochemical pathways in "sick hearts" will help to define biomarkers, therapeutic targets, and disease signatures important in cardiac health. Doll and colleagues did a principal component analysis of the 16 heart regions (inferior vena cava, right atrium, tricuspid valve, right ventricle, pulmonic valve, pulmonary artery, pulmonary veins, left atrium, mitral valve, left ventricle, aortic valve, aorta, left coronary artery, right coronary artery, interventricular septum, and interatrial septum) based on proteomic expression profiles. In the normal heart, proteomes of the cavities (right atrium, left atrium, right ventricle, left ventricle, interventricular septum, and interatrial septum), vessels (aorta, pulmonary artery, right coronary artery, left coronary artery, inferior vena cava, and pulmonary veins), and valves (tricuspid valve, mitral valve, aortic valve, and pulmonic valve) differed – there was one major cluster of highly and coexpressed proteins for each of the three anatomical areas (proteins with a high expression in the cavities were enriched in terms of cardiac muscle contraction, Z disc, and sarcomere organization when compared to that of the vessels and valves). Mitochondria and respiratory electron transport chain were also enriched in the cavities. Differences were found between atria and ventricles with mitochondrial proteins being more abundant in ventricles. Large arteries had higher levels of cytoskeleton proteins and proteins involved in cell junction. These findings emphasize the notion that the different areas of the heart vary from a protein standpoint. With disease and aging, there will be changes as well as the proteomes (complete set of proteins in a cell) are dynamic and change in response to stimuli. The effect of CO on proteomes needs further study and will help identify the role of CO in cardiac health. Reprogramming of cells at the protein level to induce repair and healing of damaged tissue is the goal of this technical innovation. The exact role of CO in this dynamic cardiac physiology has not been well defined, but determination of constituent proteins in cardiac health and "sickness" helps refine our understanding of its significance and is being further studied.

Metabolomics refers to the complete set of low molecular weight compounds in a sample (i.e., components of the heart) and helps in determining a sample's profile of these compounds at a specified time under specific environmental conditions. These compounds are the substrates and by-products of enzymatic reactions and have a direct effect on the phenotype of the cell. Small-molecule metabolite profiling in complex cardiac systems continues to advance rapidly. Cardiovascular diseases are a primary target in metabolomics due to the role metabolic alterations play in their pathogenesis and evolution and have been applied from patient risk stratification to myocardial infarction and heart failure. The heart's metabolic status reflects its health status, is quantifiable, represents the biochemical state in health and disease, and provides a tool for precision medicine whose goal is to use advanced diagnostic testing to customize an individual's medical treatment according to their specific omic profile. Relying on analytical chemistry techniques and advanced computational methods to characterize complex biochemical mixtures, metabolomics is used to analyze solids, liquids, and gases and can be performed *in vivo* or *in vitro*. The primary workhorses are nuclear magnetic resonance spectroscopy, gas chromatography mass spectrometry, and liquid chromatography MS and are metabolomics tools that have been used to study clinical cardiovascular disease. The massive amount of information that has been and will be harvested present ongoing management challenges – scaling throughput and technical capacity for metabolomics approaches, bioinformatic and chemoinformatic tools for handling large-scale metabolomics data, methods for elucidating the biochemical structure and function of novel metabolites, and strategies for determining the true clinical relevance of metabolites observed in association with cardiovascular disease outcomes. However, small-molecule metabolite profiling has already identified biomarkers and metabolomic signatures in cardiac disease.[128-138] Metabolomics – used to diagnose disease, understand disease mechanisms, identify novel drug targets, customize drug treatments and monitor therapeutic outcomes – will help define CO effects on the normal heart and with different pathologies and can be qualified and quantified.

Summary

While CO has been seen as a toxic agent, we now know that it is important for overall health. The effects of endogenous CO on the heart are extensive and can be cytoprotective, therapeutic, and/or regenerative. Implicit to its role in cardiac health and cardiovascular disease, CO affects cells at the molecular level. Prerequisite to normal cardiac function, CO interactions with other gasotransmitters require further study and clarification to determine the role of CO in myocardial health and disease. Cardiac biochemical changes affected by CO are being targeted as points of therapy. Therapeutic roles are being determined, endogenous production upregulated, exogenous administration studied, and simplified delivery systems refined as changes induced by CO are important in determining cardiovascular health. Advancing our understanding and interpretation of data that has already been and will be derived will help clinicians treat and manage cardiac diseases and thereby help with improving cardiovascular clinical outcomes.

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