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Cardiac Dys-Synchronization and Arrhythmia in Hyperhomocysteinemia

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Abstract

Although cardiac synchronization is important in maintaining myocardial performance, the mechanism of dys-synchronization in ailing to failing myocardium is unclear. It is known that the cardiac myocyte contracts and relaxes individually; however, it synchronizes only when connected to one another by low resistance communications called gap junction protein (connexins) and extra cellular matrix (ECM). Therefore, the remodeling of connexins and ECM in heart failure plays an important role in cardiac conduction, synchronization and arrhythmias. This review for the first time addresses the role of systemic accumulation of homocysteine (Hcy) in vasospasm, pressure and volume overload heart failure, hypertension and cardiac arrhythmias. The attenuation of calcium-dependent mitochondrial (mt), endothelial and neuronal nitric oxide synthase (mtNOS, eNOS and nNOS) by Hcy plays a significant role in cardiac arrhythmias. The signal transduction mechanisms in Hcy-induced matrix metalloproteinase (MMP) activation in cardiac connexin remodeling are discussed.

Keywords

Heart failure; calpain; hypertension; vascular remodeling; ECM; calcium channel; tachycardia; bradycardia; arrhythmia; LVH; fibrosis; MMP; TIMP; integrin; connexin; contraction; relaxation; endothelial myocyte coupling; smooth muscle; PAR; PPAR; NOS; metabolomics; sudden cardiac death; NMDA; GABA

INTRODUCTION

Cardiovascular disease is the number one cause of cardiac deaths. In order to treat the disease and not just manage the cardiac patients, it is inevitable to understand the mechanisms (genetic as well as environmental) of vascular disease. We have been demonstrating as to what causes the stroke, cardiac arrhythmia, and vascular embolism, spasm and dementia in low risk population, i. e. what is the hidden risk factor? One of the causes of these devastating diseases is the de-arrangement in the homocysteine (Hcy, a non-protein amino acid) metabolism and its clearance. Since vascular cells lack a significant component of homocysteine clearance, they are the prime target of its complications, therefore, in brain it causes microvascular blood brain barrier leakage and edema leading to vascular dementia, a precursor of Alzheimer's disease. In blood vessels, homocysteine

causes damage to the inner lining of the vessel wall, the endothelium, unequivocally, and creates an acute coronary syndrome and stroke situations. In addition, in the chronic and acute structural heart disease, hyperhomocysteinemia instigates cardiac muscle hypertrophy and arrhythmia. This suggests that hyperhomocysteinemia contributes to microvascular permeability, dementia, embolism, spasm, cardiac arrhythmias and stroke.

One fourth of the deaths in the developed nations are sudden cardiac deaths (SCD), which are caused by profound malfunctioning in the electrophysiological properties of the heart called cardiac-arrhythmias. Arrhythmias refer to abnormal rhythm of the heart also called as dys-arrhythmias which can occur in atria and ventricles.

Defective metabolism of the essential amino acid methionine, results in overt hyperhomocysteinemia (HHcy) or situational HHcy (after a methionine load), and is established as an independent risk factor for atherosclerotic heart diseases. It is very-well appreciated that atherosclerosis, angina, valvular heart disease, weakened heart muscles (i.e., cardiomyopathy), blood clots, thyroid abnormalities contribute to the malfunctioning of the electrical conductance in the heart.

The important aspect in understanding the cellular and molecular mechanisms in Hcy-mediated arrhythmias has been the identification and modulation of the key components of abnormal electrophysiological conductance in heart such as induction of matrix metalloproteinases, altered expression of gap junctional proteins (i.e., connexins), malfunctioned cross talk between global and mitochondrial calcium, differential expression of nitric oxide synthase (NOS) isoforms (i.e., nNOS, eNOS, iNOS) and other stress responses like volume and pressure overload. The aim of this review is to have broader view on the various aspects by which Hcy alters electrophysiological properties of the heart.

CONGESTIVE HEART FAILURE CAUSED BY HOMOCYSTEINE (Hcy)

Elevated levels of Hcy are an important independent risk factor for the development of cardiovascular diseases, stroke, thrombosis and dementia (Hashimoto *et al.*, 2003; Tyagi, 1999; Lomindaze *et al.*, 2006). Systemic accumulation of Hcy instigates mild to severe hyperhomocysteinemia. There are five ways by which Hcy is metabolized and accumulated in the plasma and tissues: by 1) a methionine rich protein diet; 2) de-methylation of methionine and methyl transferase (MT) hyper activity; 3) a vitamin B12/folate, methyltetrahydrofolate reductase, and methyl synthase (re-methylation) deficiency; 3) a heterozygous/homozygous trait for cystathione β synthase activity, B6 deficiency (transsulfuration); and 5) renovascular stenosis, volume retention (kidney disease, hypertension and diabetes) (Fig. 1). In addition, acute and chronic inflammatory conditions also increase Hcy and redox stress by generating reactive oxygen species (ROS) in systemic vascular beds. This leads to endothelial dysfunction, vascular hypertrophy, vasoconstriction and systemic hypertension. The systemic and vasoconstriction also increase after load in the heart, causing pressure overload heart failure. In addition, vasoconstriction reduces (kidney) filtration and causes volume retention which in turn increases preload and causes overt heart failure due to volume overload and further amplifying the ill-effects of hyperhomocysteinemia (Fig. 1).

SIGNALS IN MMP INDUCTION IN CHF AND HHcy

Matrix metalloproteinases (MMPs) are members of a family of zinc containing endopeptidases that share structural domains, but differ in substrate specificity, cellular sources, and inducibility. Extra cellular matrix (ECM) remodeling in cardiovascular diseases largely depends on MMP-2, and -9 (Rao *et al.*, 1993; Sato & Seiki, 1993; Davies *et al.*, 1993; Woessner 1998). MMPs are latent in normal myocardium; however, during CHF and

hyperhomocysteinemia MMPs are activated (Tyagi *et al.*, 1998). Hcy induces endothelial dysfunction (Miller *et al.*, 2000; 2002) and impairs microvascular endothelial cell function *in vivo* (Ungravi *et al.*, 1999). These effects are mediated by Hcy-induced MMP-9 expression (Hunt & Tyagi 2002). The activation of MMP-9 is important in vascular remodeling; however the upstream regulatory pathways that control MMP-9 secretion are less well understood. It is very well appreciated that Hcy potentiates thrombin generation which has been identified as a potential activator of protease activated receptor (PAR). Thrombin activates PAR-1, -2 and -3, while PAR-2 is activated by trypsin and tryptase. Recent studies identify PAR family members in the vasculature and heart. PAR activation is also linked to the structural remodeling of the vasculature and the myocardium. Recent studies from our lab (Moshal *et al.*, 2005) provided the novel evidence that in a aortic-vena cava fistula (AV fistula) model for chronic heart failure, active myocardium triggers PAR-1 and administration of cardiac inhibitor of tissue metalloproteinase-4 (TIMP-4/CIMP) normalized the PAR-1 expression and ameliorated the endothelial-myocyte uncoupling by decreasing oxidant-mediated proteolytic stress. It was also reported that MMP-1, rather than any of the other proteases, targets PAR-1 on breast cancer cells to confer promigratory and proinvasive phenotype (Boire *et al.*, 2005). They demonstrated that MMP-1 directly triggers PAR-1-mediated calcium signals in MCF7 cells, and both MMP-1 inhibitors and PAR-1 antagonist inhibited cell migration *in vitro* and tumor growth in nude mice. While PAR-1 is known to mediate the relaxation, thrombin-induced vasoconstriction is likely to be mediated by PAR-1 (Bhattacharya & Cohen, 2000). Also PARs are functionally coupled to the nitric oxide release and thrombin receptor appears to modulate both vasodilator and constrictor responses, while PAR-2 is linked only to vasodilatation (Magazine *et al.*, 1996). PAR-4 G-protein coupled activity plays a role in cardiac remodeling and influences the functional outcome at sites of cardiac inflammation through a Src tyrosine kinase mechanism (Sabri *et al.*, 2003). Additionally, rapid release of vascular MMP-2 by thrombin could contribute to short term processes where thrombin is involved, such as regulation of platelet aggregation and vascular reactivity. Vascular tyrosine kinase/phosphatase likely modulates this action of thrombin to prevent exaggerated platelet aggregation, thrombosis, and vasospasm (Frenandez-Patron *et al.*, 1999). These reports suggest a link between the action of tyrosine kinase and vascular contraction and remodeling.

The above observations prompted to hypothesize the involvement of Hcy-mediated activation of MMP/PAR signal axis in calcium signaling and increased secretion of MMP-9, leading to cardiovascular remodeling. Now, the question arises, what are the downstream signal pathways that regulate MMP-9 expression? The MMP-9 gene contains AP-1 transcription factor binding sites in its promoter region (Fini *et al.*, 1998; Vu & Werb, 1998). And the AP-1 transcriptional activity is regulated by MAPK/ERK-1/2 pathway. Hcy activated the ERK pathway in vascular smooth muscle cells leading to atherosclerosis (Brown *et al.*, 1998). The role of MAPK and NF- κ B has been suggested in MMP-9 release during tissue remodeling in asthma by triggering PAR-2 (Vliagoftis *et al.*, 2000). All of these findings have prompted us to hypothesize the possible involvement of MAPK/ERK signal cascade in Hcy-mediated MMP-9 induction. We have shown that Hcy-induced MMP-9 is regulated by ERK-1/2 pathway and further extended the understanding of the mechanism involved in Hcy-mediated ERK activation (Moshal *et al.*, 2006). Hcy induces an alteration in calcium dynamics (Zhang *et al.*, 2001; mujumdar *et al.*, 2000) in different cell systems. Ca^{2+} overload activates the cytosolic calcium-dependent cysteine proteases (calpain) which lead to cardiac ischemic injury (Wang & Yuen, 1994). There is a scant literature on signaling pathways responsible for calpain regulation. Recently, it is observed that calpains and ERK are the most likely events within a single pathway and suggested calpain as upstream activators of ERK-1/2 during neuronal injury (Veeranna *et al.*, 2004; Glading *et al.*, 2004). These observations prompted us to hypothesize that Hcy activates calpains by altering calcium dynamics and is regulated by ERK pathway. The question arises is there any

connection between calpain and MMPs? The possibility of connection between the calpain and metalloproteinases had been suggested previously (Chapman & Wei, 2001). Recently it has been observed that the calpain/calpastatin system mediates MMP-2 and -9 mRNA expressions of leukemic cells leading to their invasiveness (Popp *et al.*, 2003). To date, it is not clear how the calpain is regulating MMP secretion. We hypothesize that calpain cross talks with PI3/AKT (PKB) pathway and modulates MMP secretion. Understanding the MMP/PAR axis and the mechanism of MMP regulation by calpain will lead us to develop a potential therapeutic tool (anti-proteolytic) against inflammatory diseases and cardiovascular remodeling (Figs. 2 and 3).

MECHANISM OF PRESSURE AND VOLUME OVERLOADED HEART FAILURE

Hcy increases asymmetric dimethyl arginine (ADMA) and decreases L-arginine, in part, by inhibiting DDAH, an enzyme that converts ADMA to L-arginine (Postovit *et al.*, 2002; Selley 2004). Hcy inhibits COX (cyclooxygenases), cytochrome P450 epoxygenases (CYP) and LOX thereby decreasing prostaglandins PGJ₂, EETs and leukotriene LB₄.

The COX, cytochrome P450 epoxygenases and LOX decrease prostaglandins PGJ₂, EETs and leukotriene LB₄. Various reports project the importance of arachidonic acid (AA) metabolism in atherosclerotic related events (Fleming *et al.*, 2001), however there is a scant literature supporting the modulation of AA metabolomics in the setting of HHcy. It has been reported that the Hcy modulates AA pathway and predisposes to atherosclerotic events (Signorello *et al.*, 2002; Leoncini *et al.*, 2006). These observations prompted us to look into the role of AA metabolites in modulation of the Hcy/MMP-9 signal axis and to delve into the possible molecular mechanisms. Our unpublished study demonstrates that Hcy attenuates CYP2J₂ protein levels and activates MMP-9, a process which involves inactivation of PI3-kinase/AKT signal and an NF- κ B-dependent mechanism. Furthermore, the P450 epoxygenase transfection or administration of 8,9-EET activates PI3-Kinase/AKT pathway and attenuates Hcy-induced MMP-9, in part by the inhibition of NF- κ B and IKB α activation. Thus, our findings provide new insight into understanding the molecular mechanisms for MMP-9 regulation in HHcy and specifically propose a role for CYP epoxygenase and its AA metabolites in the modulation of Hcy/MMP-9 signals. This information may be helpful in developing a novel therapeutic approach to HHcy-associated vascular dysfunction.

In vascular remodeling, MMPs are induced *via* PAR and ERK-dependent pathways. Hcy-mediated MMP-9 activation sheds the integrin and increases focal adhesion complex (fibrosis) by activating kinase pathway (Fig. 4). Fibrosis or alterations in connexins lead to conditions promoting re-entrant arrhythmias. In the stress conditions like pressure and volume overload, the latent MMPs are activated. During volume overload PAR-1 is induced (Moshal *et al.*, 2005), causing systolic failure by increasing ERK, MMP-2, -9 and TIMP-3 and decreasing TIMP-4. The pressure overload induces shedding, causing diastolic failure by increasing FAK, MMP-2, TIMP-3, and TIMP-4 (Fig. 5).

Hcy AND CARDIAC ARRHYTHMIAS

Cardiomyocytes are branched, multinucleated, contractile cells that communicate *via* gap junctions. Gap junctions are derived from small connecting tunnels known as connexin. Six connexins join together to form a connexon that extends outward and from each adjacent plasma membrane forming a channel that allows accurate and quick generation and sweeping of action potentials across the heart. A specific MMP that may have detrimental effect on the heart is MMP-9. Furthermore, degradation of gap-junction proteins caused by

the activation of MMP-9 can alter the conduction of electrical impulses leading to tachycardia.

Microinjection of homocysteine in the brain stem increases blood pressure (Nosaka *et al.*, 2002). This can lead to brain micro vascular spasm. Coronary micro vascular spasm can also cause myocardial ischemia (Sun *et al.*, 2002). This suggested that focal micro vascular fibrillation can obstruct the blood flow. Previous studies from our lab (Hoit *et al.*, 2002) and others (Xu *et al.*, 2004) have suggested role of ECM remodeling in atrial fibrillation and arrhythmias. Altered connexin-43 expression produces arrhythmia substrate in heart failure (Poelzing & Rosenbaum, 2004). Previously we have demonstrated the positive correlation in increased connexin-43 expression and degradation in human heart failure which is associated with MMP-9 activation (Hunt *et al.*, 2002). Hcy induces connexin-43 in endothelial cells (Li *et al.*, 2002). However, paradoxically the connexin-43 is nitrated and is translocated to the mitochondria (Li *et al.*, 2002), suggesting the alteration in connexin-43 expression which causes production of arrhythmogenic substrates.

Although the high levels of Hcy and HHcy are associated with vascular seizure, dementia, and arrhythmias; the mechanism of Hcy-mediated cardiac arrhythmias is unclear. In CHF latent MMPs are activated and levels of Hcy are elevated. Hcy instigates the activation of latent MMPs in part by increasing oxidative stress. The studies suggest that Hcy increases heart rate in normal and hypertensive animals and decrease in Hcy levels slow down the heart rate. In failing human myocardium connexin (Con)-43 is degraded. In *ex vivo*, MMP-9 activation degrades con-43. These novel findings led us to suggest that cardiac arrhythmias is in part regulated by the increased oxidative stress, activation of MMP and degradation of Con-43 secondary to HHcy (Figs. 6 and 7).

Systemic vascular dysfunction (endothelial dysfunction and vascular hypertrophy) is the hall mark of systemic hypertension. Elevated levels of Hcy cause endothelial cell injury and vascular smooth cell proliferation. The studies from our lab have dissected Hcy-induced oxidative stress mechanisms and suggested that mitochondrial redox state during HHcy is regulated in part by the modulation of key oxidative stress markers (i.e., iNOS, NADPH oxidase (NOX) and thioredoxin) (Tyagi *et al.*, 2005). Furthermore, these observations suggested that endothelial dysfunction and vascular hypertrophy in hypertension is due in part to the increased expression of calcium-dependent NOS in mitochondria causing oxidative stress secondary to HHcy (Fig. 7). The ablation of calcium-dependent eNOS and nNOS genes increases heart rate and induces tachycardia (Mohan *et al.*, 2001). Hcy decreases both eNOS and nNOS, which suggest that the Hcy causes cardiac arrhythmias by decreasing availability of neuronal nitric oxide.

One of the challenges in preventing SCD lies in identifying individuals at highest risk for SCD within a lower-risk population. The increased serum Hcy has been identified as a risk factor for SCD resulting from coronary fibrous plaques (Burke *et al.*, 2002). Although both ischemia and reperfusion induce arrhythmia, only reperfusion-induced arrhythmia is sensitive to NMDA-R1 blockade (D'Amico *et al.*, 1999). This may suggest that arrhythmia in high cardiac output is influenced by circulating factors and is mitigated by NMDA-R1 blockade. In addition, HHcy is an important risk factor for the development of vasospasm, thromboembolism and stroke (Loscalzo 2002). The GABA-A receptor agonist (muscimol) mitigates the coronary vascular events and Hcy antagonizes GABA-A receptor and increases vascular complications.

SUMMARY

Sudden cardiac death is widely acknowledged to be electrophysiological in nature and is considered to be due to ventricular fibrillation. Ventricle fibrillation is one mechanism of SCD. Cardiac remodeling is characterized by increased QRS duration, deepening of in Q wave and elongation in T wave inversion. Although the treatment by ACE inhibitor reverses the QRS duration and T wave inversion, however, the deepening in Q wave still persist, in part due to fibrosis/scar formation which malfunctions the electrical conductance in the heart. The signaling mechanism by which Hcy activates MMP, PAR, oxidative stress and ERK is addressed. The exciting aspect of this review is the role of matrix remodeling in the cardiac electrophysiology which leads to arrhythmias. The activation of MMPs causes degradation in gap junctional proteins (con-43) and leads to alteration in conductive pathway between inside and outside of the cell. The role of Hcy in cardiac conduction during volume overload heart failure is documented. These observations are still in their infancy and need to be explored before drawing any conclusion. Much remains to be learned regarding how the induction of MMPs is fully interpreted by the cardiomyocytes cell system leading to the modulation of heart conductance. These and other queries need to be answered to decipher the mechanisms underlying the clinically important arrhythmias which will pave the way for potential therapeutic approaches.

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Fig. 1. Systemic role of homocysteine (Hcy): Mild hyperhomocysteinemia and acute/chronic inflammatory responses increase redox stress. This leads to vasoconstriction, increasing afterload, causing pressure overload heart. Vasoconstriction also leads to reduced renal filtration and volume retention further amplifying the Hcy, increasing preload and overt heart failure due to volume overload.

Fig. 2.

Hcy-induced signal transduction: Hcy –induced pertussistoxin-sensitive GPCR. Hcy induces protective pathway *via* PI3K and AKT. Hcy induces apoptotic pathway *via* calpain and MMP, leading to vascular remodeling. Hcy induces dephosphorylation of AKT leading to the activation of FKHRL1 (FOXO-3a) transcription factor and transcribing thioredoxin interacting protein (TXnip) causing oxidative burst.

Fig. 3.

Mechanism of Hcy-redox-receptor signaling: Hcy-mediated electron transfer in the mitochondria during energy production, this leads to by products, oxidant. In peroxisome, via calcium, IP_3 and phosphatase, Hcy inactivates $PPAR$ and RXR , catalase and SOD which in turn activates $NF-kB$ and NOX . Hcy uncouples $eNOS$ by decreasing BH_4 which increases peroxynitrite and nitrotyrosine.

Fig. 4.

Hcy increases ADMA and decreases L-arginine. Hcy inhibits COX, CYP and LOX therefore decreases PGJ_2 , EETs and LB_4 . This leads to vascular remodeling. Activated MMP induces PAR and ERK. Hcy shed the integrin and increases focal adhesion complex (fibrosis) by activating kinase cascade.

Fig. 5.

Pressure and volume overload activate latent MMP. Volume overload induces PAR-1 causing systolic failure by increasing ERK, MMP-9, -2 and TIMP-3 decreasing TIMP-4. The pressure overload induces integrin shedding which causes diastolic failure by increasing FAK, MMP-2, TIMP-3 and -4.

Fig. 6. Remodeling during MI, hypertension, Hcy load and LVH triggers ion fluxes, activation of CNS, MMPs, receptor and drugs which leads to energetics and ROS production. Activation of ion-channel, gap junctions especially remodeling in cxn-40, 43, 45 causes fibrosis and scar leading to ventricular tachycardia, fibrillation and SCD.

Fig. 7.

Metabolomics of Hcy: During gene and protein methylation, s-adenosyl homocysteine (SAH) is generated by methyl transferase and methionine. SAH hydrolase generates Hcy. DZA blocks SAHH otherwise Hcy induces NOS, NADH oxidase and decreases thioredoxin in mitochondria. These events lead to endothelial dysfunction, vascular remodeling and hypertension. In the heart these events activate MMP-9 that degrades connexin and instigates tachycardia.

Table 1

The Differential Role of Hcy in Various Mechanistic Pathways of Diseases

Pathway	Hcy
Endothelial growth retardation/disease	High
Smooth muscle proliferation/hypertrophy	High
Vascular permeability	High
GABA-Receptor	Antagonist
NMDA-Receptor	Agonist
Arrhythmia	High
Inflammation	High
Hypertension	High
Alzheimer's disease	High