Modulating cGMP levels as therapeutic drug targets in cardiovascular and non-cardiovascular diseases

FZ Mónica1,2, F Murad2*, K Bian2*

Abstract
Introduction
Cyclic guanosine monophosphate (cGMP) signaling plays a critical role in physiological homeostatic processes such as smooth muscle tone in vascular and non-vascular tissues, platelet activity, cardiac contractility, renal function and fluid balance, as well as cell growth. The cGMP signaling consists of cGMP-generating guanylyl-cyclases, protein kinases, phosphodiesterases, endopeptidases, ion channel and efflux transporters and hence substances that increase cGMP levels are important targets for the treatment of cardiovascular and non-vascular-related diseases. Studies of the 90’s established endothelium dysfunction as one of the major causes of cardiovascular diseases and therapeutic strategies that benefit NO bioavailability have been applied in clinical medicine extensively. Recently, the basic and clinical studies of cGMP regulation through either activation of particulate guanylyl cyclase (pGC) and soluble guanylyl cyclase (sGC) or inhibition of cyclic nucleotide phosphodiesterase type 5 (PDE5) have resulted in effective therapies for congestive heart failure, pulmonary hypertension, erectile dysfunction and more recently for benign prostatic hyperplasia (LUTS-BPH). This review will focus on basic and clinical research, showing how sGC, pGC and PDE regulations lead to beneficial effects on cardiovascular (heart failure and pulmonary hypertension) and non-cardiovascular disorders. The use of nitrovasodilators to increase cGMP formation and their use in ischemic heart diseases and heart disease have been extensively revised and thus it will not be addressed in the present review.

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. Also animal care was in accordance with the institution guidelines.

Clinical importance of the particulate guanylate cyclase activation in cardiovascular diseases
Since our earliest studies in the 1970’s, seven different isoforms of particulate guanylate cyclase (pGC) have been found to date in vertebrates, GC-A through GC-G. Among them, GC-A appears to be the most widely expressed throughout the body and was found in different cell types such as smooth muscle, platelet, endothelium, vascular cells, macrophages, neutrophils, eosinophils, mast cells, and certain cells in the central nervous, respiratory, and immune systems. The pGC activation is essential for the inhibition of PDEs. Alterations of cGMP-dependent pathways play an important role in cardiovascular and other related diseases, such as hypertension, pulmonary hypertension (PH), congestive heart failure (CHF), erectile dysfunction (ED) and lower urinary tract symptoms (LUTS) secondary to benign prostatic hyperplasia (LUTS-BPH).

Introduction
The second messenger cyclic 3’-5’ guanosine monophosphate (cGMP) is one of the key mediators of cell signaling and serves as an internal messenger to regulate a variety of physiological processes, including vascular1 and non-vascular smooth muscle relaxation2,3,4, natriuresis5, platelet function6, neutrophil adhesion7, sperm motility8, fluid and ion secretion9 and cancer cell proliferation10,11. The intracellular levels of cGMP are controlled by its formation mainly due to the activation of soluble guanylyl cyclase (sGC) and particulate guanylyl cyclase (pGC)12,13,14,15 and by its degradation by cyclic nucleotide phosphodiesterases (PDEs) activities 16.

Cytoplasmatic levels of cGMP may also be modulated nonenzymatically by multi-drug resistant proteins type 4, 5 and 8 (MRP 4, MRP5, MRP 8, respectively), which pumps cGMP or cAMP out of the cell17. The physiological effects of cGMP are exerted through the activation of cGMP-dependent protein kinases (PKG), cyclic nucleotide-gate ion channels and the activation and/or inhibition of PDEs. Alterations of cGMP-dependent pathways play an important role in cardiovascular and other related diseases, such as hypertension, pulmonary hypertension (PH), congestive heart failure (CHF), erectile dysfunction (ED) and lower urinary tract symptoms (LUTS) secondary to benign prostatic hyperplasia (LUTS-BPH).

Conclusion
The discovery of the NO-cGMP pathway revolutionized the comprehension of pathophysiological mechanisms involved on cardiovascular diseases. However, considering the expression “from bench to bedside”, the therapeutic alternatives that target NO-cGMP did not immediately follow the biochemical and pathophysiological revolution since few therapeutic options have been proven effective and released on the market for the treatment of cardiovascular disorders.

*Corresponding author
Email: bcmkxb@gwu.edu

1 Department of Pharmacology, Campinas, Brazil
2 Department of Biochemistry and Molecular Medicine, Washington, United States

Competing interests: None declared.

All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Licensee OAPL (UK) 2014. Creative Commons Attribution License (CC-BY)

nerves, vessels, kidney, lung, intestine, testis, heart and adipose tissues.

GC-A is stimulated by atrial (ANP) and B-type natriuretic peptides (BNP), whereas GC-B is activated by C-type natriuretic peptide (CNP)\(^{14}\). ANP is a 28-amino acid with a disulphide bond between two cysteine residues at positions 7 and 23 and released by atrial granules. BNP is a 32-amino acid polypeptide, first isolated from brain homogenates, although a greater proportion of circulating BNP is thought to come from ventricles\(^{22}\). CNP is a 22-amino acid peptide and binds preferentially to GC-B, which is highly expressed in bone, lung, brain, heart and ovary\(^{23}\) and macrophage\(^{24}\).

ANP, BNP and CNP are released as a pro hormone proANP1-126, proBNP1-108, proCNP1-126, respectively, which are then enzymatically cleaved into mature and more effective forms of ANP1-28, BNP1-32 and CNP1-22.

Nephrilysin is a metalloendopeptidase and is involved in the metabolism of enkefalin, tachykinins, natriuretics and chemotactic peptides. The physiological effect of ANP, BNP and CNP can be attenuated by either increasing nephrilysin activity or reducing their bioavailability.

Hence increasing the concentration of natriuretic peptides through nephrilysin inhibition represents a potential therapeutic approach for cardiac, vascular and renal protection\(^{25}\).

ANP and BNP possess diuretic, natriuretic and hypotensive activity. Cardiomyocytes release ANP and BNP in response to volume and pressure overload, which increase cardiac transmural distending pressure. Circulating natriuretic peptide levels are highly correlated with the severity of systolic heart failure and both BNP and N-terminal fragment of BNP (NT-proBNP) concentrations are valuable biomarkers in the management of heart failure, since its levels are raised more than ANP in left ventricular overload\(^{26}\). NT-pro-BNP is derived from proteolysis of pro-BNP1-108 and consists of 76 amino acids. Both BNP and NT-proBNP are released in the plasma in equimolar quantities and the latter is an alternative choice in monitoring heart failure due to its better stability (> 3 days) and longer half-life (60-120 min) when compared to BNP (24 hours and 21 min, respectively)\(^{27,28}\).

In a prospective, multicenter trial, circulating BNP concentrations were determined to diagnose and evaluate the severity of patients with heart failure based on American Heart Association classification compared to the patients without cardiovascular symptom (BNP=9.29 pg/mL, n=473), the blood levels of BNP in heart failure class 1 through IV were 83.1 pg/mL (49.4-137 pg/mL, n=73), 235 pg/mL (137-391 pg/mL, n=135), 459 (200-871 pg/mL, n=141) and 1119 pg/mL (728-1300 pg/mL, n=60), respectively, showing a high positive correlation\(^{29}\). It is important to emphasize that sex, age, body mass index, hypernatremia, myocardial ischemia, sympathetic nervous system activation, tachycardia and endocanal substances (endothelin, angiotensin II, glucocorticoids and thyroid hormones) are conditions that can affect BNP/NT-proBNP synthesis and clearance\(^{30}\) and should be taken into consideration when BNP/NT-proBNP levels are used as diagnostic tools.

Nesiritide, a 32-amino acid peptide, is an exogenous form of BNP manufactured by using recombinant DNA technology and approved by FDA in 2001 for use in the treatment of heart failure. However, despite its favorable cardiorenal properties, such as natriuresis, diuresis, inhibition of renin and aldosterone, BNP is a potent vasodilator and may decrease renal perfusion, thus compromising renal function in heart failure patients\(^{31}\).

Thus, there is a need for novel natriuretic peptides that favors renal function. LCz696 is a combination drug consisting of valsartan and AHU-377, which inhibits concomitantly nephrilysin and the angiotensin receptor. In patients with chronic heart failure, LCz696 increased plasma cGMP levels while decreasing NT-proBNP and plasma aldosterone levels\(^{24}\). The PARADIGM-HF trial of Novartis showed that chronic heart failure patients with reduced ejection fraction who received LCz696 lived longer without being hospitalized than those who received enalapril, an angiotensin-converting-enzyme (ACE) inhibitor, only\(^{32}\) (Figure 1).

CNP mainly works in a paracrine manner and possess less diuretic and natriuretic effects since it lacks a C-terminus amino acid extension\(^{33}\). CNP plays a role in the suppression of neo-intima formation after intimal lesions of rat carotid arteries\(^{34}\). In cultured cardiac myocyte hypertrophy, CNP had anti-hypertrophic, anti-fibrotic and anti-proliferative effects and hence may be useful in preventing cardiac remodeling after myocardial infarction\(^{35}\). CD-NP (cenderitide) is a novel chimeric natriuretic peptide developed by Mayo Clinic consisting of full length CNP fused with the carboxyl tail Dendroaspis Natriuretic Peptide (DNP). DNP is an ANP analogue purified from the snake venom that activates GC-A. CD-NP is a dual activator of GC-A and GC-B, with higher affinity at GC-B than GC-A (Figure 1).

In normal anaesthetized dogs CD-NP elevated cGMP, enhanced glomerular filtration, reduced cardiac filling pressures and suppresses the renin-angiotensin system\(^{36}\). In healthy volunteers CD-NP (17.5 ng/Kg/min) increased plasma cGMP, urinary cGMP excretion, urinary sodium excretion and urine flow within 4 hours of infusion\(^{37}\). In a randomized, double blind, placebo-controlled clinical trial with patients of chronic heart failure (ejection fractions≤40%), cenderitide (20 ng/Kg/min) increased cGMP levels without altering on blood pressure or heart rate. Aldosterone and ANP levels were reduced and glomerular filtration rate increased by using of cenderitide\(^{38}\).

In a Phase II trial sponsored by Capricor Therapeutics, intravenous infusion at 1.25, 2.5 and 3.75 ng/Kg/min appeared well tolerated with a dose-dependent decrease on systolic blood pressure compared to placebo. Serum creatinine levels in the 1.25 and 2.5 groups improved relatively to the placebo. These findings led the authors to conclude that CD-NP was well tolerated and preserved renal function relative to placebo. However, more clinical studies need to be carried out to further evaluate the efficacy of CD-NP.
out to larger groups of patients in order to evaluate its effectiveness and safety.

Guanylyl cyclase type C (GC-C) is localized almost exclusively in the epithelial cells of small intestine, but is also present in liver, uterus, kidney and pancreas, serves as the receptor for guanylin, uroguanylin, lymphoguanylin and heat-stable enterotoxin (STa). In mammals, uroguanylin and guanylin are quite different in primary structures with uroguanylin possessing two acidic amino acids near N-terminus and an internal asparagine residue. Instead, guanylin have a conserved aromatic amino acid, tyrosine or phenylalanine. The bacterial STa peptide resembles uroguanylin more than guanylin in primary structure, because they possess an internal asparagine making them highly resistant to proteolytic attack by endoproteases such as chymotrypsin. Since GC-C expression is presented mainly on intestinal mucosa, the majority of studies are focused on the role of GC-C pathway on cancer, diarrhea and obesity mechanisms (for review see ).

Clinical importance of the nitric oxide-soluble guanylate cyclase activation in cardiovascular diseases

Nitric oxide (NO) can be considered as an intracellular second messenger with autocrine, paracrine, and endocrine effects. Nitric oxide is biosynthesized by the action of nitric oxide synthases (NOS). Once formed, it diffuses into the cell to activate its intracellular receptor - soluble guanylate cyclase (sGC), which is a heterodimer protein consisting of two subunits, alpha (α) and beta (β).

The most commonly studied isoform is the α1β1 protein, although α2β2 subunits have also been identified. Nitric oxide binds to heme of sGC to form a 6-coordinate complex, which is rapidly converted to a 5-coordinate ferrous nitrosyl complex and thus catalyzing the conversion of guanosine triphosphate (GTP) into cGMP.

Recently, two classes of compounds that activate sGC have been approved by FDA and European Union Agency for treating two forms of pulmonary hypertension, thus, the therapeutic strategy by targeting sGC-cGMP signaling has again regained attention. It has been known from our studies in the 1970’s that nitroglycerin and other nitrovasodilators acted by forming nitric oxide and increasing sGC activity and cGMP formation.

The World Health Organization divides PH into five groups: 1) pulmonary arterial hypertension (PAH), that includes idiopathic PAH, familial PAH and PAH associated with other conditions such as connective tissue disease and congenital heart disease; 2) pulmonary hypertension with left heart failure, where mitral valve disease or long-term high blood pressure can cause left heart failure and PH; 3) PH associated with lung diseases, such as chronic obstructive pulmonary disease and interstitial (IN-ter-STISH-al) lung diseases; 4) PH due to pulmonary embolism in the lungs or blood clotting disorders and 5) PH caused by other diseases such as blood disorders (polycythemia vera, essential thrombocythemia), systemic disorders (sarcoidosis, vasculitis), metabolic disorders (thyroid and glyco-gen storage diseases). In all groups, the average increased pressure in the pulmonary arteries is 25 mmHg or higher. The pressure in normal pulmonary arteries is 8–20 mmHg at rest.

Inhaled NO is used to treat newborn with PAH (WHO group 1). Low doses of inhaled NO at 20 ppm for 4 hours and then 6 ppm for 20 hours resulted in improved oxygenation without systemic effects and decreased systolic blood pressure in 9 newborn infants with persistent pulmonary hypertension.

License OAPL (UK) 2014. Creative Commons Attribution License (CC-BY)

Critical review

The ability of inhaled NO to increase systemic oxygen levels was confirmed in multicenter randomized controlled studies of term and near-term babies with hypoxemia and PAH\textsuperscript{50,59}. In adults with severe PAH, pulmonary vascular resistance was reduced significantly after inhaled NO and after prostacyclin (24 μg/h), whereas systemic vascular resistance was not affected by inhaled NO\textsuperscript{60}. In an open, prospective, randomized, controlled trial, the combination of inhaled NO and oxygen improved pulmonary hemodynamics better than inhaled NO alone\textsuperscript{61}. However, inhaled NO has only been formally approved by the FDA and the European agency (EMEA) for clinical use in the treatment in term and near-term neonates with hypoxemia and pulmonary hypertension.

A significant proportion of adults with PAH do not respond to inhaled NO, likely because of sGC impairment\textsuperscript{62} or irreversible pulmonary disease such as fibrosis. Organic nitrates are not effective for PH because their long-term use leads to tolerance and upon discontinuation the development of serious rebound pulmonary hyper-tension.

Furthermore, pulmonary smooth muscle is less sensitive to nitrovasodilators-induced relaxation than vascular smooth muscle\textsuperscript{63,64}. It is worth noting that in patients with CTEPH (WHO 4), pulmonary endarterectomy (a surgery to remove old blood clots from the pulmonary arteries) is a therapeutic choice to restore pulmonary haemodynamics. However, in the cases of CTEPH it is deemed ineffective and those who develop persistent PH after surgery are eligible for using PAH-specific pharmacotherapy such as prostanoids, endothelin receptor antagonists, PDE5-inhibitors and more recently sGC stimulators.

Three completed randomized controlled trials in patients with inoperable CTEPH have used prostanoids\textsuperscript{65}, PDE-5 inhibitors, sildenafil\textsuperscript{66} and an endothelin-1 receptor antagonist, bosentan\textsuperscript{67}. Only bosentan demonstrated a positive therapeutic effect on hemodynamics, although no improvement was observed in exercise capacity.

Further trials are needed to ascertain the role of pharmacotherapy on patients with CTEPH. The role of the sGC stimulator riociguat (BAY 63-2521) in patients with CTEPH will be addressed below. In patients with PH-associated with left heart disease (WHO group 2) the role of PAH-specific drugs is controversial and not well studied in large randomized controlled trials. However, recent studies suggest that acute\textsuperscript{68,69} and chronic\textsuperscript{70,71} administration of oral sildenafil reduced pulmonary artery pressure and pulmonary vascular resistance without significant changes in systemic arterial pressure.

It has been known for many years that PDE5 inhibitors can augment the accumulation of both cAMP and cGMP in most tissues including pulmonary tissues and blood vessels to increase their effects\textsuperscript{69,52,72,73}. As for patients with PH-associated lung diseases (WHO 3) there exist few clinical evidences showing the efficacy of PDE-5 inhibitors. Sildenafil improved pulmonary hemodynamics at rest and during exercise in 20 patients with chronic obstructive pulmonary disease (COPD)-associated PH\textsuperscript{74}, whereas in another study sildenafil had no effect on stroke volume or exercise capacity in 9 patients with COPD-associated PH\textsuperscript{75}.

The phosphodiesterases (PDEs) are a family of enzymes that hydrolyze 3',5'-cyclic nucleotidemonophosphates into their respective 5'-monophosphates. PDE 5, PDE 6 and PDE 9 are selective for cGMP\textsuperscript{16} (Figure 2). An experimental ovine fetal model for perinatal pulmonary hypertension of the neonate was characterized by altered pulmonary vasoreactivity and structure. Infusion of PDE-5 inhibitors, dipyridamole and zaprinast lowered pulmonary resistance by 55 and 35%, respectively in hypertensive animals. Moreover, PDE5 activity was 150% higher in hypertensive fetal lambs in comparison with healthy animals\textsuperscript{76}. In chronically hypoxia mice with disruption of gene encoding eNOS sildenafil reduced pulmonary arterial pressure less than in wild-type controls and failed to decrease right ventricular hypertrophy\textsuperscript{77}.

In the same model where GC-A was blunted, PDE5 inhibition reduced...
pulmonary arterial pressure, right ventricular hypertrophy and pulmonary vascular remodeling with less efficacy than wild-type animals, suggesting that NO or natriuretic peptides also influence to the vasodilator response to sildenafil by enhancing cGMP levels. Patients with idiopathic PH (n=9, WHO 1), PAH (n=2, WHO group 1) or PH due to left heart diseases (n=2, WHO 2) were given sildenafil, inhaled NO or sildenafil+inhaled NO. The decrease in pulmonary vascular resistance was similar with inhaled NO (-19 ± 5%) and sildenafil (27 ± 3%), whereas sildenafil+inhaled NO (-32 ± 5%, P<0.003) was more effective than inhaled NO. In addition, sildenafil and sildenafil+inhaled NO increased cardiac index. Ten PAH patients with normal left ventricular function were included in a prospective randomized, placebo-controlled cross over study to evaluate the short-term effect of sildenafil. Patients in the sildenafil group have and improvement of 6-minute walk distance from 163.9 to 266.7 m (P<0.0005) and a decrease in the Borg dyspnea index (from 5.2 to 3.6, P<0.01) and pulmonary artery systolic pressure (from 80.8 to 55.3, P<0.05). In another double blind, placebo-controlled study, patients with PAH received placebo or sildenafil (20, 40 or 80 mg) for 12 weeks. The 6-minute walk increased from baseline in all sildenafil groups.

All sildenafil doses reduced the mean pulmonary-artery pressure, showing the efficacy of sildenafil in improving exercise capacity and haemodynamics. These observations provided the rationale to target PDE5 inhibition for the treatment of pulmonary hypertension with or without agents that enhance cGMP formation such as natriuretic peptides and/or nitrovasodilators. As mentioned above, activating sGC has been approved as a new therapeutic method for pulmonary hypertension. sGC activation can be reached by two mechanisms: the sGC stimulators (BAY 41-2272, BAY 63-2521, BAY 41-8543) are dependent on the presence of the reduced (ferrous-Fe²⁺) prosthetic on sGC heme and works synergically with NO, whereas sGC activators (BAY 58-2667, BAY 60-2770 and HMR 1766) preferentially and effectively activate sGC when it is oxidized (ferric-Fe³⁺) or in a heme-free state (Figure 2). Pulmonary arterial hypertension is associated with endothelial dysfunction and thus low bioavailability of NO, sGC stimulators or activators overcome such limitations, since these substances activate sGC in a NO-independent manner. In a phase I study of 58 healthy male volunteers, oral riociguat (BAY 63-2521) was well tolerated. In a phase IIa study in 19 patients with diagnosis of PAH (WHO 1), distal chronic thromboembolic (WHO 4) or PH associated with mild to moderate interstitial lung disease (WHO 3), riociguat demonstrated hemodynamic efficacy and favorable tolerability to a greater extent than inhaled NO. After a 2.5 mg dose of riociguat, mean arterial pressure fell by 14%. In patients with CTEPH (WHO 4) and PAH (WHO 1) mean arterial blood pressure decreased 4.5 mmHg from baseline after riociguat treatment (1 to 2.5 mg 3 times daily for three weeks). Dyspnea and a 6-minute walking distances showed significant improvement.

In a phase III, multicenter, randomized, double-blind, placebo-controlled study, 261 patients with CTEPH (WHO group 4) or persistent pulmonary hypertension after surgery (WHO group 4) patients who received riociguat (n=173) had a mean increase of 39 m, whereas placebo (n=88) group a mean decrease of 6 m on 6-min walk distance test. Pulmonary vascular resistance decreased and increased in riociguat and placebo group, respectively. Riociguat also significantly reduced NT-proBNP levels. In October 2013 riociguat was the first-in-class sGC stimulator approved by FDA for PAH and CTEPH, excluding the prior use of many nitrovasodilators. In an animal model of heart failure, PDE-5 inhibition showed beneficial effects after 4 weeks of treatment compared to control, which improved contractility (end-systolic elastance 247 ± 68 vs 155 ± 71 mmHg/mL, P<0.05); prevented right ventricular dilatation (end-diastolic volume 733 ± 50 vs 874 ± 39 μL, P<0.05); reduced wall stress (peak wall stress 323 ± 46 vs 492 ±62 mmHg, P<0.05) and partially preserved exercise tolerance (running distance -33 ±15 vs -62 ± 12%, P<0.05), respectively.

In mice exposed to chronic pressure overload induced by transverse aortic constriction, sildenafil reduced chamber and myocyte hypertrophy and improved in vivo heart function. Patients with congestive heart failure, sildenafil (10 mg) decreased pulmonary artery systolic (-21.6%) and diastolic (-31.8%) pressure and arteriolar resistance (-36.9%), without affecting ejection fraction, cardiac index, wedge pulmonary pressure and blood capillary volume, suggesting that sildenafil ameliorates pulmonary haemodynamics. In a fixed-dose double-blind, randomized, placebo controlled, two-way crossover study, the effect of sildenafil (50 mg) was investigated in 23 men with chronic heart failure. Sildenafil reduced significantly heart rate, systolic and diastolic blood pressure before and after cardiopulmonary walking and exercise test. Chronic treatment with sildenafil (50 mg, 8/8 hours) for 4 weeks improved maximal oxygen uptake, ventilator efficiency and oxygen uptake kinetics in patients with chronic heart failure with an ejection fraction of 28 ± 6% in comparison to placebo. However, a multicenter, double-blind, placebo-controlled, parallel-group, randomized clinical trial of 216 outpatients with heart failure (ejection fraction ≥ 50%, elevated BNP and reduced exercise capacity), sildenafil (20 mg 8/8 hours for 12 weeks followed by 60 mg 8/8 hours for 12 weeks) did not result in significant improvement in exercise capacity or clinical status.

Solute guanylate cyclase stimulator (riociguat, BAY 63-2521) and activator (cinaiguat, BAY 58-2667) are also being investigated in PH patients with left heart disease (WHO group 2). In patients with acute decompensated heart failure ritodrine was more effective than placebo (P<0.0001). In patients with PAH treated with riociguat, HbA1c levels were significantly decreased (P<0.05). In patients with PAH treated with riociguat, HbA1c levels were significantly decreased (P<0.05).

Competing interests: None declared. Conflict of interests: None declared. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.
heart failure, infusion of cinaciguat (50-400 mg/h, 6 hours) led to significant reductions in pulmonary capillary wedge pressure, mean right arterial pressure, mean pulmonary artery pressure, pulmonary vascular resistance and systemic vascular resistance, while increasing heart rate and cardiac output93. In patients with heart failure with preserved left ventricular ejection fraction and pulmonary hypertension, riociguat (2 mg) significantly increased stroke volume, decreased systolic blood pressure and right ventricular end-diastolic area92. Whether sGC stimulators or activators are effective in heart failure requires more clinical trials with a larger number of patients.

Clinical applications of NO-cGMP regulators through vascular-regulated mechanisms

Lower urinary tract symptoms (LUTS), benign prostatic hyperplasia (BPH) and erectile dysfunction (ED) are common conditions in elderly males and the prevalence increases with aging. Clinical and basic research data suggest that the incidence of LUTS, BPH and ED are higher in individuals with vascular risk, such as hypertension, diabetes, hyperlipidaemia and obesity. Besides, many clinicians consider ED as an early symptom of vascular damage and should be used as a diagnosis for the identification of cardiovascular diseases93.

Alterations of the NO-cGMP pathway, enhancement of Rho-associated protein kinase (ROCK), contractile signaling, autonomic adrenergic hyperactivity and pelvic atherosclerosis might decrease pelvic arterial blood flow, leading to arterial insufficiency, smooth muscle relaxation and contractility alterations and thus bladder94, prostate95 and penis96 dysfunctions. Since there exist clinical evidence of the interrelation between ED and BPH-related LUTS, substances that alter the NO-cGMP signaling pathway are an alternative for the treatment of LUTS-BPH-ED.

Nitric oxide is the principal mediator in penile erection and it can be released from the action of endothelial or neuronal nitric oxide synthase in “nitrinergic neurons”. Rajfer et al.97 was the first group to demonstrate that electrical field stimulation (EFS) induced frequency dependent relaxation, which was inhibited and enhanced, respectively, in the presence of inhibitors of NOS (N-nitro-L-arginine and N-amino-L-arginine) or sGC inhibitors (methylene blue) and PDE (M&B 22,948), suggesting that NO is an important mediator of nonadrenergic noncholinergic neurotransmission.

Few years latter studies have shown that the magnitude of relaxation induced by EFS98 or by NO-donors99 was reduced in men with vascular impotence in comparison with controls, thus providing evidence that lower levels of NO may be responsible for erectile dysfunction. Since 1990 many studies have been published showing that alterations on NO-pathway, such as low NO-bioavailability and/or oxidation of sGC is the main cause of erectile dysfunction. The first-in-class selective phosphodiesterase type 5 (PDE5) inhibitor, was zaprinast introduced in the early 1980’s. Subsequently, Viagra (sildenafil citrate) was introduced in 1998 for the treatment of ED. The impact of sildenafil has stimulated academic, clinical and industrial research to better understand the nature of sexual function and develop better treatment and management for sexual dysfunctions such as ED. However, patients with ED and high cardiovascular risk do not respond to PDE5 inhibitor treatment. Nonresponding patients showed a significant higher severity and duration of ED, higher level of arterial insufficiency and higher endothelial apoptosis100. Thus, new treatments options are required to fulfill the medical need in those men with greater endothelial damage.

Soluble guanylate cyclase stimulators and activator constitute a new therapeutic option for ED, when NO formation or bioavailability is decreased by oxidative stress or when PDE-inhibitors are no longer effective. Soluble guanylate cyclase stimulator, BAY 41-2272 relaxed pre-contracted corpus cavernosum from rabbit101,102, human101,102 and mice103, which was reduced in the presence of L-NAME, a NOS inhibitor or ODQ, a sGC inhibitor. In human corpus cavernosum from PDE5 inhibitor nonresponders co-incubation with BAY 60-4552 (sGC stimulator) and vardenafil (PDE-5 inhibitor) induced significantly greater relaxation compared with either compound alone104. In rats with cavernous nerve injury both vardenafil (0.03 mg/Kg) and BAY 60-4552 (0.03 and 0.3 mg/Kg) increased intracavernous pressure (ICP) and their combination provided synergic response on ICP105. Intracavernous (ic) administration with sGC activator BAY 60-2770 increased ICP and this response was enhanced by ODQ (2 mg/Kg, ic)106. To date sGC stimulators has been approved for the treatment of pulmonary hypertension. Whether sGC stimulators and activators have any role on erectile dysfunction, controlled, randomized, placebo-control studies should be carried out in order to ascertain the safety, efficacy and cost-effectiveness of these drugs in urolologic disorders.

Lower urinary tract symptoms (LUTS) secondary to benign prostatic hyperplasia (BPH) is a condition that impacts the quality of life in elderly men and characterized by increased frequency of urinary and urgency with or without nocturia. Therapeutic management for LUTS-BPH is aimed to relax bladder and/or prostate smooth muscle and to inhibit prostatic cell growth. There is an increasing interest in agents that increase cGMP levels for the treatment of LUTS-BPH, since there exist a dense nitrinergic innervation in the transition zone of the human prostate, fibromuscular struma, glandular epithelium and blood vessels107. Recently, FDA approved tadalafil for the treatment of LUTS-BPH. PDE5 is expressed in vessels, bladder, urethra, and prostate smooth muscle, but not in epithelium.

In human prostate, SNP induced concentration-dependent relaxation and was significantly potentiated by tadalafil (30-60 nM). The combination
of tadalafil and tansulosin, an alpha1-adrenoceptor blocker, reduced significantly EFS-induced contraction in prostate and bladder neck\textsuperscript{109}. In prostatic smooth muscle, but not in bladder cells, NO-donor, sodium nitroprusside (SNP) and sGC stimulator, BAY 41-8543 showed a concentration-dependent antiproliferative effect, which was enhanced in the presence of vardenafil\textsuperscript{109}.

The efficacy of tadalafil as monotherapy\textsuperscript{110,111,112,113} or in combination with alpha-adrenoceptor blockers\textsuperscript{114,115} or 5-alpha reductase inhibitors\textsuperscript{116} for the treatment of LUTS-BPH has been demonstrated in several randomized, placebo controlled clinical trials.

**Conclusion**

The discovery of the NO-cGMP pathway by our lab in the 1970’s revolutionized the comprehension of pathophysiological mechanisms involved on cardiovascular diseases.

However, considering the expression “from bench to bedside” we concluded that therapeutic alternatives that target NO-cGMP did not immediately follow the biochemical and pathophysiological revolution, since few therapeutic options have been proven effective and released on the market for the treatment of cardiovascular disorders such as recombiant brain natriuretic peptide (nesiritide), inhaled NO, PDE-5 inhibitors (sildenafil, tadalafil and vardenafil) and more recently sGC stimulator (riociguat). Initially, the scientific community was reluctant to engage in beta2 integrins on human neutrophils activates the monomeric GTPases Rap1 and Rap2 and promote adherence. J Biol Chem. 2006 Nov; 281(46):35008-20.


13. Waldman SA, Rapoport RM, Fiscus RR, Leitman DC, Chang LY, Murad F. Regulation of particulate guanylate cyclase by atropineptins: relation between peptidase structure, receptor binding, and enzyme kinetics. Biochim

---

**Abbreviations**

NO, nitric oxide; GTP, guanosine triphosphate; cGMP, cyclic 3’-5’ guanosine monophosphate; sGC, soluble guanylyl cyclase; pGC, particulate guanylyl cyclase; PDE’s, phosphodiesterases; PKG, protein kinase G; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-natriuretic; NT-BNP, N-terminal fragment of BNP; CD-NP, chimeric natriuretic peptide; GC-A, guanylyl cyclase A; GC-B, guanylyl cyclase B; GC-C, guanylyl cyclase C; ROCK, Rho-associated protein kinase; PH, pulmonary hypertension; PAH, pulmonary arterial hypertension; CHF, chronic heart failure; CTEPH, chronic thromboembolic pulmonary hypertension; COPD, chronic obstructive pulmonary disease; LUTS, lower urinary tract symptoms; BPH, benign prostatic hyperplasia; ED, erectile dysfunction; EFS, electrical field stimulation.

**References**


13. Waldman SA, Rapoport RM, Fiscus RR, Leitman DC, Chang LY, Murad F. Regulation of particulate guanylate cyclase by atropineptins: relation between peptidase structure, receptor binding, and enzyme kinetics. Biochim
40. Guerrant RL, Hughes JM, Chang B, Robertson DC, Murad F Activation of intestinal guanylate cyclase by heat-stable enterotoxin of Escherichia coli: studies of tissue specificity, potential...
55. MURAD F. Nitric oxide signalling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? Recent Prog Horm Res. 1998;53:43-59; discussion 59-60.

Licensee OAPL (UK) 2014. Creative Commons Attribution License (CC-BY)

Effects in iNopErable Forms of chronic Thromboembolic pulmonary hypertension Study Group. Bosentan for treatment of inoperable chronic thromboembolic pulmonary hypertension: BENEFIT (Bosentan Effects in iNopErable Forms of chronic Thromboembolic pulmonary hypertension), a randomized, placebo-controlled trial. J Am Coll Cardiol. 2008 Dec; 52(25):2127-34.


73. Murad F. Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? Recent Prog Horm Res. 1998; 53:43-59; discussion 59-60.


