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Stimulators of Soluble Guanylyl Cyclase: Future Clinical Indications

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ABSTRACT

Background: Soluble guanylyl cyclase (sGC) is expressed in mammalian cytoplasm and catalyzes the synthesis of the second messenger guanosine 3′,5′-monophosphate (cGMP) involved in important physiological functions such as relaxation of vascular smooth muscle, inhibition of platelet aggregation, modulation of inflammation, and control of vascular permeability. sGC is the intracellular receptor for nitric oxide (NO) and the active moiety in traditional organic nitrate therapy, recently as an inhalant in the intensive care unit and experimentally in improving microcirculatory flow in shock. However, dysfunction of the heme moiety on sGC occurs in a number of cardiovascular diseases, which reduces NO effectiveness.

Methods: In this review, we examine animal studies and early clinical trials on agents that can directly stimulate sGC and may have future clinical application in cardiovascular disease and in perioperative care.

Conclusions: Animal and early clinical studies have shown that sGC stimulator agents have great promise for treating cardiopulmonary disorders and may also have a role in modulating the inflammatory response observed in perioperative care.

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INTRODUCTION

The receptor for nitric oxide (NO) in vascular smooth muscle is the heterodimetric enzyme, soluble guanylyl cyclase (sGC).[1,2] However, NO binding to sGC requires a reduced (ferrous) heme moiety for activation of the enzyme.[3,4] Activated sGC catalyzes formation of guanosine 3′,5′-monophosphate (cGMP) from guanosine triphosphate, and cGMP plays a major role in regulating vascular tone as well as inhibiting platelet aggregation, modulating inflammation, and controlling vascular permeability (Figure 1).[5-9] The NO-sGC-cGMP pathway serves in important physiologic roles, but pathway signaling can be impaired by scavenging of NO by free radicals, uncoupling of NO synthase (NOS), removal or oxidation of the heme moiety of sGC, downregulation of sGC, and increased phosphodiesterase (PDE) activity, which reduces NO bioavailability (Figure 1).[10] Cardiovascular diseases such as systemic and pulmonary hypertension, coronary artery disease, congestive heart failure (CHF), peripheral vascular disease, diabetes, and atherosclerosis and noncardiovascular diseases such as sepsis are associated with impaired NO-sGC-cGMP signaling.[11-14]

Perioperative injury produces marked microcirculatory alterations as a result of early systemic inflammatory responses and tissue hypoperfusion.[15-19] Although goal-directed hemodynamic therapy may decrease the incidence of perioperative complications and hospital length of stay, optimal management continues to be debated.[20-23] Recent investigations demonstrated that low-dose NO therapy improves microvascular perfusion following injury.[24-27] However, a major limitation in the current use of organic nitrates is the development of acute tolerance.[28-30] Agents that could improve bioavailability of NO in vascular diseases by sensitizing sGC are the focus of this review.

CURRENT ORGANIC NITRATE THERAPY

Glyceryl trinitrate (nitroglycerin; GTN) and amyl nitrite have been used in the treatment of angina pectoris and heart failure for more than 140 years.[31,32] Today, commonly used NO donor agents—isosorbide

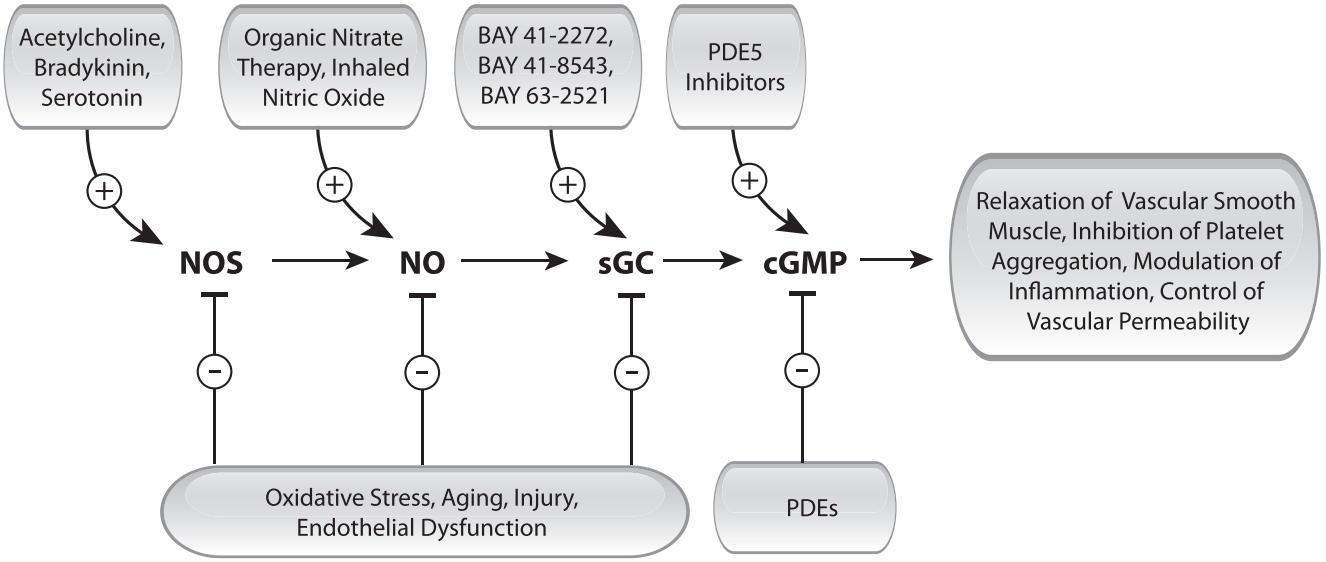

Figure 1. NO-sGC-cGMP pathway. Agonists such as acetylcholine, bradykinin, and serotonin encourage the production of nitric oxide (NO) though activation of nitric oxide synthase (NOS). Organic nitrate therapy and inhaled NO can stimulate soluble guanylyl cyclase (sGC) when the heme moiety on sGC is in a reduced (ferrous) state. Novel non-NO-dependent sGC stimulators can increase the activity of sGC. Activated sGC stimulates production of cyclic guanosine 3′,5′-monophosphate (cGMP) that through downstream mechanisms elicit relaxation of vascular smooth muscle, inhibition of platelet aggregation, modulation of inflammation, and control of vascular permeability. Phosphodiesterases (PDEs) break down cGMP. Oxidation of the heme moiety in sGC from stress, aging, injury, and the development of tolerance from organic nitrates reduces sGC activation. PDEs break down cGMP, whereas PDE5 inhibitors prevent the breakdown of cGMP. (Adapted with permission from Boerrigter G, Burnett JC Jr. Nitric oxide-independent stimulation of soluble guanylate cyclase with BAY 41-2272 in cardiovascular disease. *Cardiovasc Drug Rev.* 2007 Spring;25(1):30-45.)

dinitrate, isosorbide-5-mononitrate, and GTN—are effective in reducing ventricular preload.[33-36] These agents also decrease pulmonary and systemic vascular resistances but higher doses than those needed for reducing ventricular preload are required.[37-42]

Studies have shown GTN bioactivation requires the presence of thiols or sulfhydryl-containing compounds.[1,43-45] Repeated administration of GTN depletes sulfhydryls and quickly produces tolerance to GTN, limiting its use to episodic care.[45-48] Chronic administration of organic nitrates does not improve mortality following acute myocardial infarction, and continuous administration of GTN has induced endothelial dysfunction.[46,49,50] However, in microcirculation abnormalities observed in acute heart failure and sepsis, administration of GTN improves tissue perfusion.[24,26,51] The development of agents that could stimulate sGC to endogenous NO (bioavailable NO) or to low levels of exogenous NO donors may provide a novel therapeutic in the management of microcirculation abnormalities.

STIMULATION OF sGC BY NON-NO COMPOUNDS

In the 1990s, investigators observed that a benzylindazole, YC-1, originally designed as an inhibitor of platelet aggregation (Figure 2), also increased intracellular cGMP concentrations by 10-fold. This finding stimulated research in sGC regulation.[52,53] Subsequent studies showed that stimulation of sGC by YC-1 was NO independent and independent of biotransformation.[54,55] The combination of sodium nitroprusside (SNP), an NO donor, with YC-1 was found to be synergistic.[56] These findings suggested that non-NO-donor compounds that stimulate sGC could have a therapeutic role in cardiovascular disease when NO formation and bioavailability are impaired or when tolerance to NO donors has developed.[56-62] Because YC-1 could not be formulated as an oral preparation, limiting its potential for chronic therapy, a series of pyrazolopyridinylpyrimidine derivatives based on the YC-1 structure (Figure 2) led to the next generation of sGC stimulators for study in animals and then in early clinical trials.[57-59]

Animal Studies

CHF and Systemic Hypertension. The prevalence of CHF is increasing and despite medical therapy continues to be associated with high morbidity and mortality.[63] In a canine model of CHF, BAY 41-2272 (Figure 2) reduced mean arterial pressure and pulmonary arterial pressure with increased cardiac

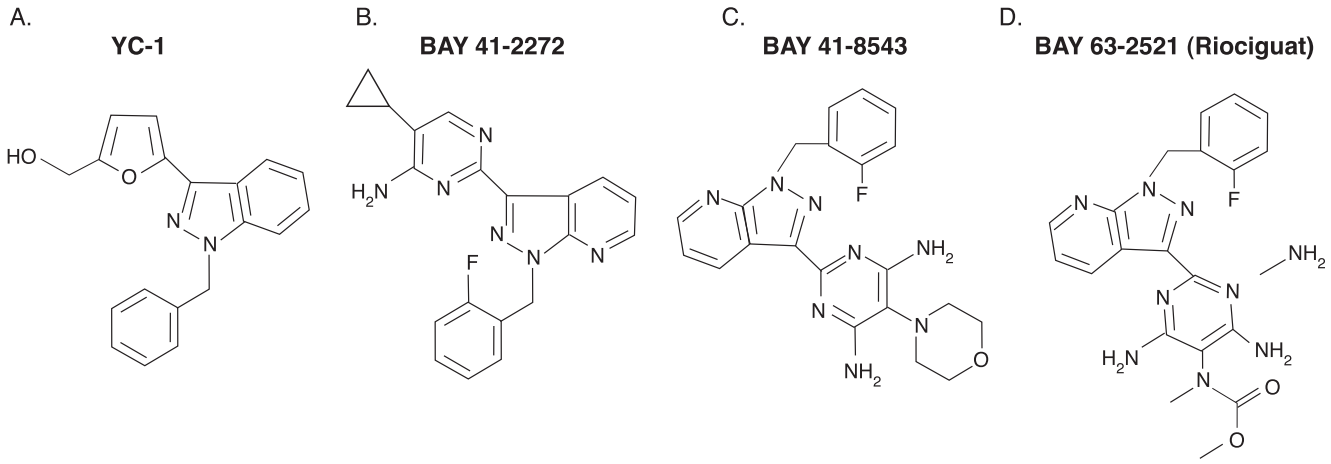

Figure 2. Chemical structures of soluble guanylyl cyclase stimulators.

output. Renal blood flow increased and glomerular filtration rate was maintained. Importantly, plasma renin activity, angiotensin II, or aldosterone levels did not significantly change.[63] In a chronic low-NO model of systemic hypertension and CHF induced with chronic administration of the nonspecific NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME), BAY 41-2272 cotreatment abolished the development of systemic hypertension and attenuated cardiac hypertrophy induced by NOS inhibition in the control group. Moreover, the sGC stimulator was devoid of tolerance development.[64] In a low-NO genetic model of hypertension, the oral sGC stimulator, BAY 41-2272, lowered mean arterial pressure, demonstrated antiplatelet activity, and enhanced survival. Moreover, the sGC stimulator was devoid of tolerance.[65] In anesthetized dogs, intravenous administration of BAY 41-8543 (Figure 2) produced dose-dependent decreases in blood pressure and myocardial oxygen consumption with increases in coronary blood flow and heart rate.[66] In conscious, spontaneously hypertensive rats, dose-dependent and long-lasting (for nearly 24 hours) decreases in blood pressure with the sGC stimulator were observed with a 3 mg/kg dose. Moreover, tachyphylaxis after multiple dosages did not develop.[66] In a low-NO, high-renin rat model of hypertension, coadministration of the sGC stimulator prevented increases in blood pressure induced by L-NAME, and renal protective effects were observed. Finally, administration of BAY 41-8543 prolonged bleeding times, reduced thrombosis formation, and reduced mortality in this study.[66]

The effect of BAY 41-2272 on cardiac remodeling and fibrosis was examined in a rat model of hypertension induced by suprarenal aortic constriction.[67] Daily administration of the sGC stimulator for 14 days decreased collagen accumulation in the left ventricle and reduced angiotensin-converting enzyme messenger RNA (mRNA) and enzymatic activity.[67] The sGC stimulator BAY 63-2521 (Riociguat) (Figure 2) was investigated in high- and low-renin rat models of hypertension.[68] In hypertensive-transgenic rats treated with the NOS inhibitor L-NAME (high-renin model) and in rats with five-sixths nephrectomy (low-renin model), Riociguat treatment improved survival, as well as normalized blood pressure in both models of hypertension. In the high-renin model, Riociguat also reduced cardiac damage as suggested by lower atrial natriuretic peptide plasma levels, reduced relative left ventricular weights, and lower cardiac interstitial fibrosis. Riociguat also reduced renal damage as observed by lower plasma creatinine and urea levels, with less evidence of glomerulosclerosis and renal interstitial fibrosis. In the low-renin study, Riociguat reduced cardiac damage as indicated by lower atrial natriuretic peptide plasma levels, an observed decrease in relative left ventricular weights, smaller myocyte diameters and arterial media/lumen ratios, and reduced renal damage as suggested by improved creatinine clearance rates and decreased renal interstitial fibrosis.[68] In a Dahl salt-sensitive rat model maintained on a high-salt diet for 14 weeks, hemodynamics, biomarkers of tissue remodeling and degeneration, and mortality were assessed with Riociguat (BAY 63-2521) in two dose regimens, 3 or 10 mg/kg/d.[69] Daily administration of the sGC stimulator markedly attenuated development of systemic hypertension, improved systolic heart function, and increased survival rates from 33% to 85%. Histological examination of the heart and kidneys revealed that the sGC stimulator significantly reduced fibrotic tissue remodeling and degeneration. In the myocardium and renal cortex, mRNA expressions of the profibrotic biomarkers—tissue inhibitor of matrix metalloproteinase-1, osteopontin, and plasminogen activator inhibitor-1—were attenuated by administra-

tion of BAY 63-2521.[69] These observations suggest that sGC stimulators would be beneficial in the treatment of cardiovascular disease such as CHF and systemic hypertension.

Pulmonary Hypertension. Pulmonary arterial hypertension is a rare progressive disorder and is often fatal within 3 years without treatment.[70,71] Pulmonary hypertension is also often associated with other diseases (eg, portal hypertension, collagen vascular diseases, drug abuse, toxins, chronic thromboembolism, cancer, and right heart failure following cardiac and lung transplantation).[72-76]

In a chronic hypoxia-induced model of pulmonary hypertension, BAY 41-2272 (Figure 2) reduced right ventricular hypertrophy and pulmonary vascular remodeling.[77] In an ovine fetal model of pulmonary hypertension, chronic infusion of BAY 41-2272 produced potent sustained decreases in pulmonary arterial pressure; however, with infusion at higher rates, systemic arterial pressure also decreased.[78] In an acute model of pulmonary hypertension, inhalation of either BAY 41-2272 or BAY 41-8543 (Figure 2) in awake lambs produced selective pulmonary vasodilation. Moreover, BAY 41-8543 also enhanced the magnitude and prolonged the duration of the vasodilator response to inhaled NO.[79,80] However, in contrast to the above findings, in an intact-chest rat model, intravenous administration of BAY 41-8543 during baseline or under controlled conditions produced small decreases in pulmonary arterial pressure, increases in cardiac output, and large dose-dependent decreases in systemic arterial pressure.[81] When pulmonary and systemic arterial tone was elevated with the thromboxane receptor agonist U46619, BAY 41-8543 produced larger dose-dependent decreases in pulmonary and systemic arterial pressure.[81] Analyses of the percent decreases in pulmonary and systemic arterial pressures in response to BAY 41-8543 were not different. These data suggested that the sGC stimulator had similar vasodilator activities in the pulmonary and systemic vascular beds in the intact-chest rat.[81]

In a second investigation of responses to BAY 41-8543 conducted in elevated tone conditions induced by the NOS inhibitor L-NAME, again pulmonary and systemic arterial pressures decreased following administration of the sGC stimulator. However, the vasodilator responses in L-NAME–treated animals caused by BAY 41-8543 were lower than the vasodilator responses caused by BAY 41-8543 in U46619-infused animals.[81] A comparison of vasodilator responses to BAY 41-8543 at the same level of pulmonary arterial pressure indicated that the decreases in pulmonary arterial pressure in response to the sGC stimulator were reduced by more than 50% in L-NAME–treated animals.[81] These findings suggest that responses to the sGC stimulator were NO independent and occurred in the absence of endogenous NO formation and that vasodilator responses to the sGC stimulator were present but markedly attenuated.[81]

Finally, the synergistic role of BAY 41-8543 with exogenous NO was examined in the intact-chest model with the NO donor SNP.[81] Separate administrations of BAY 41-8543 and SNP produced significant decreases in pulmonary and systemic arterial pressures, but the administration of a small dose of the NO donor in the presence of BAY 41-8543 produced a significantly greater decrease in pulmonary and systemic arterial pressures than the sum of responses to either agent when administered alone.[81] These results demonstrate that BAY 41-8543 can synergize with exogenous NO in mediating vasodilator responses. Overall, these findings suggest that these compounds have dual actions in that they can directly stimulate the native form of the enzyme and also render it more sensitive to endogenously produced NO or augment the action of NO donors.[80,82] Hence, the use of an sGC stimulator alone or in combination with an NO donor could expand the usefulness of NO therapy in the intensive care unit.[80-82]

In an animal model of acute pulmonary hypertension induced by pulmonary embolism from the infusion of plastic microspheres, treatment with BAY 41-8543 produced a 2.2-fold increase in cardiac output and a significant reduction in right ventricular peak systolic pressure.[83] Moreover, treatment with the sGC stimulator normalized oxygen saturation and serum lactate levels. Treatment with BAY 41-8543 also significantly reduced plasma free-hemoglobin content by 80%. These findings suggest that pharmacologic dilation of the nonobstructed pulmonary vasculature with the use of an sGC stimulator could be effective in the treatment of acute pulmonary hypertension from pulmonary embolism.[83]

Antiplatelet and Antiinflammatory Actions. The NO-sGC-cGMP pathway inhibits platelet aggregation and leukocyte adhesion, and the use of sGC stimulators could be clinically beneficial in thrombogenic and inflammatory disorders.[7-9] The sGC stimulator BAY 41-2272 blocked collagen-induced aggregation in washed human platelets, but GTN—which requires metabolism to an NO donor in platelets—did not block aggregation. BAY 41-2272 also significantly increased tail-bleeding times.[65] However in another study, the inhibition of platelet aggregation by BAY 41-2272 required the reduced form of sGC and the presence of NO.[84] In an endothelial NOS knockout mice model, administration of BAY 41-2272 reduced the incidence of increased leukocyte rolling and adhesion observed in this model of inflammation.[85] Adiposity has been

shown to greatly increase the risk of atherothrombotic events due to a chronic state of oxidative stress.[86] In a high-fat rat model, BAY 41-2272 inhibited platelet aggregation but was less effective in rats fed standard chow. This finding suggests that decreased NO bioavailability is present in obesity because of defects in the prosthetic heme group of sGC.[86]

Inhaled NO has decreased pulmonary vascular permeability in some, but not in all studies.[87-95] One observed complication with inhaled NO is an increase in vascular permeability due to formation of reactive oxygen species from high oxygen concentrations during ventilation and subsequent hyperoxia-induced invasion of leukocytes.[90,93,96] In an isolated ischemic-reperfusion rabbit lung model, administration of BAY 41-2272 significantly attenuated vascular leakage and suppressed reactive oxygen species generation.[97] These studies suggest that during low NO bioavailability states, BAY 42-2272 could modulate platelet aggregation, leukocyte activation, and vascular leakage—key components of the inflammatory response to injury.

Clinical Trials

The first sGC stimulator to undergo clinical study was BAY 41-8543 (Figure 2).[57] As expected in a Phase I study, systemic arterial pressure decreased in healthy volunteers following oral administration, suggesting efficacy of the drug in humans. However, the pharmacokinetic finding of prolonged clearance rates and the pharmacodynamic presence of active metabolites supported the development of additional sGC stimulator compounds.[57,98] Subsequently, BAY 63-2521 (Riociguat) was developed (Figure 2). When administered in 58 healthy male volunteers as a single oral dose (0.25-5 mg), researchers observed no serious adverse events.[99] Although systolic blood pressure (SBP) was not significantly affected, both mean arterial and diastolic pressures were decreased. Moreover, dose-related increases in heart rate up to $\sim$11 beats per minute were observed with the higher mg dose. The higher dose was not well tolerated; volunteers complained of headache, nasal congestion, flushing, feeling hot, orthostatic hypotension, and palpitations. Plasma levels of norepinephrine and renin were elevated, but levels of aldosterone and angiotensin II were not.[99]

A Phase I study evaluated the short-term safety profile of BAY 63-2561 (Riociguat) to determine tolerability and efficacy in patients with moderate to severe pulmonary hypertension caused by primary pulmonary arterial hypertension, distal chronic thromboembolic pulmonary hypertension, or pulmonary hypertension from mild to moderate interstitial lung disease.[100] Safety and tolerability studies involved 19 patients receiving single doses of Riociquat up to 2.5 mg. The administration of the sGC stimulator significantly improved pulmonary hemodynamic parameters and cardiac index to a greater extent than what was observed following administration of inhaled NO. Although Riociguat demonstrated no selectivity for the pulmonary circulation, mean SBP remained $>$110 mmHg.[100] The drug was well tolerated and found to be superior to inhaled NO in the pulmonary circulation.[100]

In a Phase II study, 75 patients with a diagnosis of chronic thromboembolic pulmonary hypertension or pulmonary arterial hypertension received oral Riociguat in 0.5-mg increments in 2-week intervals from 1 mg to a maximum of 2.5 mg 3 times a day titrated to systemic SBP for 12 weeks.[101] The dose of the sGC stimulator was titrated upward to a stable arterial pressure range of 90-100 mmHg or until observation of syncope or dizziness. Riociguat was well tolerated, but asymptomatic hypotension (SBP $\leq$ 90 mmHg) was observed in 11/75 (15%) patients, blood pressure normalized in 9 patients, and dose alteration was required in 2 patients.[101] Pulmonary vascular resistance significantly decreased, and the median 6-minute walking distance test scores significantly improved. Although adverse events of dyspepsia, headache, and hypotension occurred, these events were considered mild to moderate in 96% of patients, with medication discontinued in 4% of patients.[101] As a result of these initial studies, 3 additional studies have been introduced worldwide in patients with pulmonary hypertension (NCT00454558, NCT00910429, NCT00855465).

Because anticoagulants are used in patients with pulmonary hypertension, a Phase I study investigated the potential interactions between Riociguat and warfarin.[102] Steady-state plasma levels of Riociguat did not affect the pharmacokinetics of warfarin, prothrombin times, or factor VII clotting activity.[102] Although these findings are promising, repeat clinically relevant studies are needed to confirm the observed nonpharmacokinetic interaction between sGC stimulator agents and warfarin.

FUTURE CLINICAL APPLICATIONS

These sGC agents are undergoing clinical trials in patients with pulmonary hypertension and could be released in the near future. This class of agents could have additional roles in patients with endothelial dysfunction. Prior administration of sGC stimulators in a yet-to-be-defined preoperative period may allow stressed vascular beds to be more responsive to low levels of endogenous NO with associated relaxation of vascular smooth muscle, less platelet aggregation, modulation of inflammation, and control of vascular permeability. Although inhaled NO has not been shown to improve outcomes in patients with acute respiratory distress syndrome[103-106]—largely due to the development of tolerance and increased super-

oxide, endothelin-1, and peroxynitrite formation[107-110]—inhaled NO has been shown to reduce pulmonary vascular permeability in the lungs.[111] The coadministration of a low-dose sGC stimulator in the intensive care unit could make inhaled NO therapy more effective in the treatment of acute pulmonary hypertension, improve right ventricular output, and reduce pulmonary vascular permeability following cardiac transplantation or in patients who develop acute lung injury.[112-115]

Finally, an initial goal in the intensive care unit is to restore microcirculation to provide effective oxygen delivery and tissue perfusion to maintain aerobic metabolism.[116-118] Although current therapy is to optimize systemic hemodynamics, the relationship between systemic hemodynamics and microcirculatory perfusion during resuscitation is unclear.[116,119] However, studies in cardiogenic and septic shock showed that topical application of acetylcholine reversed sublingual microcirculatory disturbances, suggesting that the vascular endothelium was still responsive to vasodilators.[120,121] Because low-dose GTN has been shown to improve microcirculation abnormalities in patients with severe heart failure[24] and in patients with septic shock,[25,27] the use of sGC stimulators may allow the enzyme to also respond to low levels of bioavailable NO or to a low-dose NO donor and have benefit in the intensive care unit.

CONCLUSIONS

The NO-sGC-cGMP pathway plays an important role in the regulation of vascular tone and in the modulation of platelet aggregation, inflammation, and vascular permeability. However, endothelial dysfunction observed in cardiovascular disease inhibits this pathway. Current organic nitrate therapy is limited to episodic care and has no current role in preventive medicine. Animal and early clinical studies have shown that sGC stimulator agents offer great promise in the medical management of cardiopulmonary disorders, such as systemic and pulmonary hypertension and CHF. These agents may also have a role in modulation of the inflammatory response observed in perioperative care. These agents may allow the vascular endothelium to respond to bioavailable NO or, in selected cases, to low-dose NO donors. As these agents continue to undergo extensive animal and early Phase III trials, we look forward to their release for clinical use in cardiovascular diseases as well as in perioperative medicine.

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