Chapter

Therapeutic Applications and Mechanisms of YC-1: A Soluble Guanylate Cyclase Stimulator

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Abstract

Nitric oxide (NO) is an essential endogenous vasodilator to maintain vascular homeostasis, whose effects are mainly mediated by NO-dependent soluble guanylate cyclase (sGC) which catalyzes the synthesis of cyclic guanosine monophosphate (cGMP), a critical mediator of vascular relaxation. YC-1, a novel NO-independent sGC stimulator, was first introduced as an inhibitor of platelet aggregation and thrombosis. Accumulating studies revealed that YC-1 has multiple medication potentials to use for a broad spectrum of diseases ranging from cardiovascular diseases to cancers. In contrast to NO donors, YC-1 has a more favorable safety profile and low medication tolerance. In this chapter, we introduce canonical and pathological roles of NO, review activations, and regulatory mechanisms of YC-1 on NO-independent sGC/cGMP pathway and present the potential pharmacological applications and molecular mechanisms of YC-1.

Keywords: nitric oxide, soluble guanylate cyclase, YC-1

1. Introduction

Since the discoveries of the biological effects of NO on physiological actions mediated by cGMP, delineation of the molecular mechanism of NO actions and understanding of NO activation of guanylate cyclase (GC) and the subsequent signal processes have been greatly advanced [1]. NO can function as an intracellular messenger, an autacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects [1, 2]. It is therefore a unique simple molecule with diversified physiological functions.

2. Canonical function of NO

NO, initially known as the endothelium-derived relaxing factor (EDRF), is a gas molecule and free radical with an unpaired electron which has been shown to be involved in an ever-growing list of biological processes. NO generated in the tissue binds to major physiological target, haem moiety of GC, activating the cGMP cascade. The GC family is composed of two members including membrane-bound GC and soluble GC (sGC). Membrane-bound GC is a receptor responsive to atrial natriuretic peptide (ANP), and sGC acts as the NO sensor. NO exerts its biological

effects by activating sGC to increase the cGMP level and vascular effects known to be mediated by cGMP such as vasodilation, inhibition of platelet aggregation, and inflammatory reaction. Cyclic GMP modulates a number of signaling processes downstream of NO. The NO-cGMP cascade can be regulated by pharmacological modulation of protein kinases, phosphodiesterases (PDE), and ion channels to alter vascular tones as well as endothelial and vascular smooth muscle cell growth. Pharmacological alteration of the NO level has been a major strategy to develop therapeutic agents for cardiovascular diseases.

Deguchi and his colleagues found that GC activity in the supernatant of neuroblastoma and brain preparations were activated by L-arginine which has been identified as an endogenous activator of sGC [3]. Hibbs et al. noted the latter that the cytotoxic properties of macrophages in co-cultures with tumor cells could be enhanced with L-arginine but suppressed by N-N-methyl-arginine (LNMA), an inhibitor of nitric oxide synthase (NOS) [4]. This cytotoxicity action was accompanied by accumulation of nitrite in the conditioned medium. These important studies provide the insight to identify a pathway of L-arginine metabolism that could produce NO and nitrite.

NOS is a group of isozymes which convert L-arginine to L-hydroxyarginine and subsequently to NO and L-citrulline through cofactors including reduced nicotinamide-adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (BH4) [5]. The first NOS isoform to be identified was the neuronal NOS (nNOS or named as NOS-1) [6]. This was followed shortly thereafter by inducible NOS (iNOS), also known as type II NOS (NOS-2) [7], and then by endothelial NOS (eNOS or named as NOS-3) [8]. NOS can also be inactivated by asymmetric dimethyl arginine (ADMA), an endogenous and competitive inhibitor of NOS [9–11].

3. Pathological role of NO

NO is essential in the maintenance of vascular homeostasis including smooth muscle relaxation, inhibition of platelet aggregation, attenuation of vascular smooth muscle cell (VSMC) proliferation, neurotransmission, and immune defense [12]. Therefore, the impaired NO pathway has been implicated in endothelial dysfunction and pathogenesis of a number of diseases featuring inflammatory reaction. These include arthritis, myocarditis, colitis, and nephritis. Altered NO synthesis has been noted in selected pathologic conditions such as amyotrophic lateral sclerosis, cancer, diabetes, and neurodegenerative diseases [13, 14]. In general, physiological NO actions on target tissues are brief, reversible, and dependent on the downstream cGMP-dependent signaling events. Conversely, the pathological actions noted with excessively and sustained NO production involved NO interaction with superoxide to generate peroxynitrite, a highly reactive free radical which exhibits the toxic actions of potent oxidants. Peroxynitrite, independent of the cGMP signaling events, has been implicated in oxidative injury noted in a number of disease models [15–17]. In addition to its free radical actions, peroxynitrite inactivates prostacyclin synthase to reduce prostacyclin levels, leading to vascular dysfunction [18].

ADMA, a risk factor for cardiovascular diseases, inhibits NOS to reduce biosynthesis of NO, resulting in impaired blood flow, accelerated atherogenesis, and suppressed angiogenesis [19]. ADMA is involved in the development of endothelial dysfunction. In essential hypertension patients, the L-arginine and ADMA levels are elevated and inversely related to endothelial function [20, 21]. Endothelial function depends on the integrity of eNOS and the availability and vascular signaling of

NO. In clinical settings, endothelial dysfunction is important because it may develop hypertension and atherosclerosis and therefore is a predictor in ensuing cardiovascular diseases [22]. In hyperhomocysteinemia, an increase in ADMA has been linked to impaired vascular endothelial function. Elevated homocysteine levels exert inhibitory effects on the expression or activation of dimethylarginine dimethylaminohydrolase (DDAH) [23–27]. Two isoforms of DDAH, DDAH-1 and DDAH-2, were identified in tissues expressing nNOS and eNOS, respectively [28]. Both DDAH isoforms are expressed widely in different organs, with higher content found in the liver and kidney [29]. Similarly, endothelial dysfunction has also been found in hypercholesterolemia. Several studies indicated that hypercholesterolemia may cause a decline in DDAH activity and an increase in the ADMA level [30, 31]. Böger et al. also found that exposure of cultured endothelial cells to oxidized low-density lipoprotein (oxLDL) cholesterol resulted in ADMA accumulation in the culture medium [31]. Oxidized LDL could cause endothelial dysfunction in complex mechanisms including reduction of eNOS expression [31], to trigger endothelial apoptosis [32] and to inhibit vascular endothelial growth factor (VEGF)-induced endothelial proliferation [33]. Furthermore, oxLDL impairs NO-induced stimulation of cGMP accumulation [34]. Patients with cardiac syndrome X (CSX) have higher levels of ADMA and increased mean common carotid intima-media thickness that are ascribed to ADMA effects on NO bioavailability resulting in endothelial dysfunction and subsequently impede microvascular circulation, which are the leading mechanisms in the development of CSX [10, 35, 36]. ADMA also plays important roles in endothelial dysfunction in subjects with chronic kidney failure [9, 37, 38]. ADMA is metabolized to L-citrulline *via* the action of DDAH-1, which is highly expressed in the kidney [29]. There is a strong association between impairment of renal function and elevation of ADMA content [9, 39]. Microangiopathy-related cerebral damage (MARCD) is a cerebrovascular disease caused by arteriosclerosis in deep white matter, which includes lacunar infarction and white matter hyperintensity [40]. Arteriosclerosis in deep white matter resulting from acute and chronic ischemia is probably responsible for the development of MARCD [41]. Several potential risk factors for arteriosclerosis have been evaluated in patients with MARCD [42, 43]. NO is involved not only in regulating cerebral blood flow but also in preventing arteriosclerosis by inhibiting fibrosis and proliferation of smooth muscle cells in the arterial wall [44]. In fact, NOS inhibitors and functional single-nucleotide polymorphisms in the eNOS gene have been shown to be correlated with MARCD [44, 45]. Excessive NO production could also be a problem in the progression of the disease such as glaucoma. Increased NO generated by iNOS in astrocytes and microglia in the optic nerve head of patients with glaucoma may contribute to the optic neuropathy associated with this disease. The pharmacological use of an inhibitor of iNOS, aminoguanidine, significantly prevents the loss of retinal ganglion cells [46].

4. Novel compounds for NO-independent sGC/cGMP activation

Organic NO donors such as nitrite and nitroglycerin are successful examples in clinical practice for more than a century. However, formation of harmful intermediate, peroxynitrite, and the long-term treatment with NO donors resulting in drug resistance limit the clinical applications of NO donor compounds. To overcome these obstacles, the novel agents for triggering sGC/cGMP cascade in NO-independent manner have been developed.

A series of 1-(substituted benzyl)-3-(substituted aryl)-condensed pyrazole derivatives were synthesized and identified as class novel antiplatelet agents [47, 48]. As one of the most promising analogues, 1-benzyl-3-(5′-hydroxymethyl-2′-furyl)

indazole (YC-1) was selected for further investigation. The physiological property of YC-1 in stimulation of sGC was demonstrated by Ko and colleagues [49]. Potential regulatory mechanisms of YC-1 on cardiovascular protections were summarized in **Figure 1**. Ko et al. showed that YC-1 is an antithrombotic agent. It inhibits platelet aggregation by increasing platelet cGMP levels in an NO-independent manner. YC-1 action was noted to exert its antiplatelet effect through the activation of NO-independent sGC/cGMP pathway [50]. Nearly, all the newer generations of sGC stimulator except acryl-acrylamide family have been derived based on YC-1 as the parent compound [51]. YC-1 and its successors all require the presence of a reduced haem moiety within sGC to stimulate sGC, but they also act in synergy with NO by binding NO or iron-free precursor of haem to structurally resemble the NO-haem complex and stabilize sGC in its active configuration [52–54]. Stasch et al. also reported that YC-1 and its derivate, BAY 41-2272, bind to regulatory sites (cys 238 and cyst 243 regions) in the α1-subunit of sGC, resulting in conformational change and subsequent activation of recombinant sGC by NO-independent but haem-dependent mechanism [55]. Mulsch et al. also noted that the combined effect of nitrovasodilators and YC-1 in cultured VSMCs and isolated rabbit aortic rings reflected the direct synergistic action of YC-1 and NO on the sGC [56]. Wohlfart et al. reported that YC-1 can stimulate synthesis and release NO in endothelial cells independent of raising the cGMP content in a calcium-dependent manner [57]. In addition, YC-1 inhibits the cGMP-specific phosphodiesterase type 5 (PDE-5)

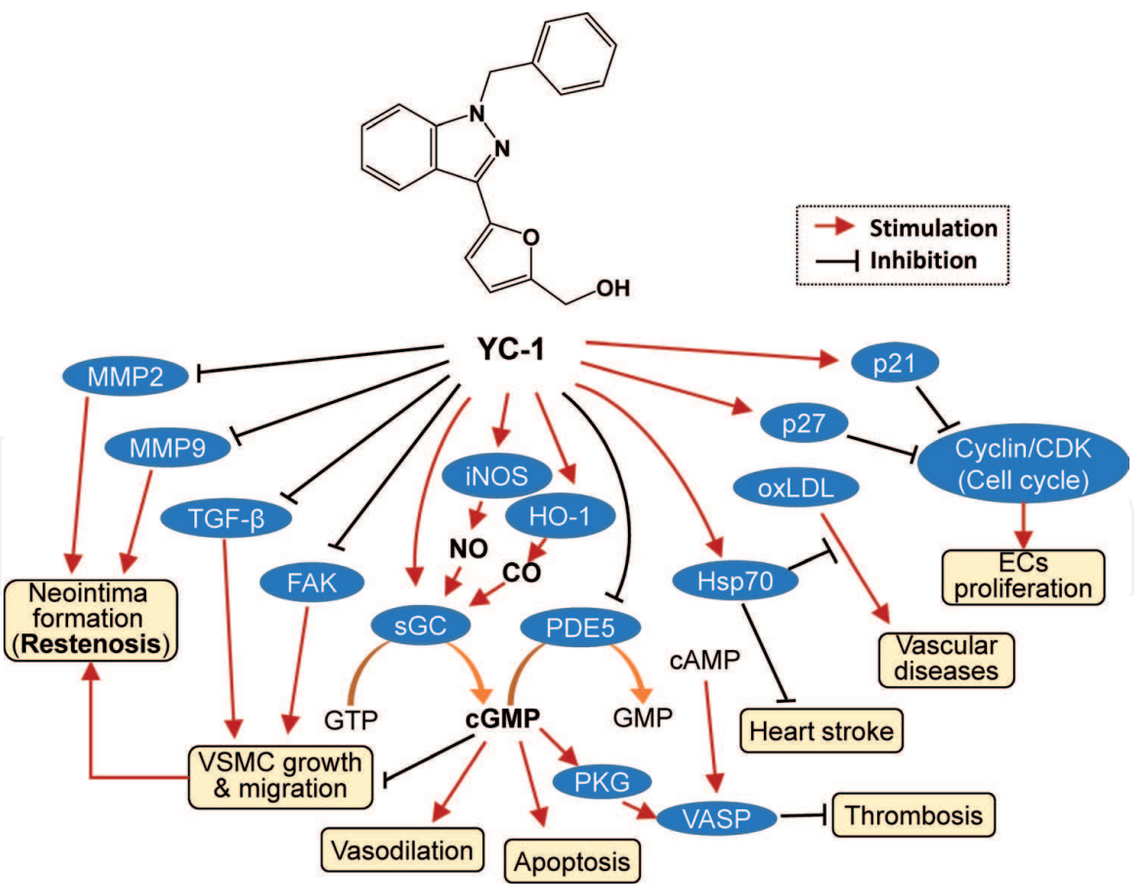

Figure 1.
Schematic overview of regulatory mechanisms of YC-1 on cardiovascular protections. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ECs, endothelial cells; FAK, focal adhesion kinase; GMP, guanosine monophosphate; GTP, guanosine triphosphate; HO-1, heme oxygenase-1; Hsp70, heat shock protein 70; iNOS, inducible nitric oxide synthase; MMP2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; oxLDL, oxidized low-density lipoprotein; PDE5, phosphodiesterase 5; PKG, protein kinase G; sGC, soluble guanylyl cyclase; TGF-β, transforming growth factor-beta; VASP, vasodilator-stimulated phosphoprotein; VSMC, vascular smooth muscle cells.

in platelets and in aortic extracts to raise cGMP levels and prolong its duration of action [58, 59]. The vasodilator-stimulated phosphoprotein (VASP) has been reported to be involved in cGMP- and cAMP-mediated antiplatelet actions [60]. Becker et al. noted that VASP is the target of YC-1 since VASP phosphorylation can be directly increased through stimulation of the cGMP/protein kinase G/VASP pathway [61].

5. Additional pharmacological activities of YC-1

In addition to the effects in antiplatelet aggregation and antithrombosis, YC-1 has been demonstrated to provide several beneficial effects including cardiovascular protections; antitumor, neuroprotective, and anti-inflammatory effects; as well as optical protections.

5.1 Cardiovascular protections

YC-1 inhibits VSMC proliferation, similar to specific guanylate cyclase inhibitors, suggesting that the antiproliferative effect of YC-1 is mediated by cGMP [62]. A similar conclusion has also been drawn by other investigators [63, 64]. As shown in **Figure 1**, NO-/cGMP-dependent processes have been suggested to modulate VSMC phenotype and the arterial response to endovascular injury [65, 66]. It has been reported that YC-1 can upregulate expression of iNOS and inducible heme oxygenase-1 (HO-1) at the transcriptional and translational level as well as stimulate sGC and cGMP production in the balloon-injured artery [63]. These results support the proposal that YC-1 can be developed as a potent new therapeutic agent for reducing restenosis *via* endogenous carbon monoxide (CO)- and/or NO-mediated cGMP-dependent processes. Wu et al. found that two important modulators, transforming growth factor (TGF)-β1 and focal adhesion kinase (FAK), responsible for VSMC proliferation and migration were reduced in content in the cultured VSMC treated with YC-1. The effect of YC-1 on preventing balloon injury-induced vascular stenosis has also been demonstrated in a rat carotid angioplasty model [64]. Liu et al. also found that YC-1 can inhibit neointima formation in balloon-injured rat carotid through suppressing the expression and actions of matrix metalloproteinase (MMP)-2 and MMP-9 [67]. YC-1 can also prevent oxLDL-mediated apoptosis by inducing heat shock protein 70 (Hsp70) expression in VSMCs suggesting its cytoprotective effect in vascular diseases [68]. Similarly, Hsp70 overexpression has also been involved in protective effect of YC-1 on heat stroke [69]. In vivo evidence shows that YC-1 and zaprinast, an inhibitor of cGMP-selective PDE, inhibit injury-induced vascular remodeling through anti-mitogenic and pro-apoptotic actions in a rat carotid artery balloon injury model [70]. Moreover, YC-1 has also been found to induce cell cycle arrest of HUVEC through upregulation of p21 and p27 protein *via* inhibition of the cyclin/cyclin-dependent kinase (CDK) system. This finding suggests that YC-1-induced antiproliferation effect in HUVEC is *via* a cGMP-independent manner [71]. Besides, the prevention effects of YC-1 on the development of hypoxia-induced pulmonary arterial hypertension (PAH), right ventricular hypertrophy (RVH), and pulmonary vascular remodeling has been clearly mentioned in animal model [72].

5.2 Antitumor effects

A growing body of evidence indicates that hypoxia-inducible factor-1 (HIF-1) contributes to tumor progression and metastasis. YC-1 inhibits HIF-1-mediated

hypoxic responses [73–76]. YC-1 enhanced radiation sensitivity by inhibiting HIF-1α expression [77]. Lau et al. also found that YC-1 suppressed both synthesis and stability of HIF-1α, *via* regulation of murine double minute (Mdm2) protein [78]. In hypoxic gastric carcinoma cell and xenograft models, low-dose YC-1 combined with glucose and insulin can effectively inhibit anaerobic glycolysis and induce hypoxia-dependent apoptosis by suppressing HIF-1α expression [79].

YC-1 also enhanced chemosensitivity of hepatocellular carcinoma cells to cisplatin through a Stat3-dependent manner [80]. Similarly, YC-1 also enhanced camptothecin toxicity by activating the caspase-8, the Bid pathway, and the mitochondria-mediated apoptotic pathway in ovarian carcinoma cell lines [81]. Additionally, it has also been found that YC-1 can suppress constitutive NF-κB activation and induce apoptosis in human prostate cancer cells [82]. YC-1 inhibited VEGF- and basic fibroblast growth factor (bFGF)-mediated ERK1/ERK2 mitogen-activated protein kinase (MAPK), AKT, and protein kinase Cα (PKCα) pathways in vitro and angiogenesis in in vivo models [83]. YC-1 arrested the cell cycle in G_0/G_1 in human hepatocellular carcinoma cells by upregulating $p21^{CIP1/WAP1}$ and $p27^{KIP1}$ expression [84]. YC-1 arrested the cell cycle at S-phase and induced apoptosis by activating checkpoint kinases in several cancer cells [85]. Similarly, YC-1 can also increase p21 protein and decrease cyclins and CDKs to induce G0/G1 phase arrest as well as activate caspases and disrupt the mitochondrial membrane potential to trigger mitochondria-dependent apoptosis in cisplatin-resistant human oral cancer CAR cells [86]. Additionally, apoptotic mechanism of YC-1 may also be mediated by activating JNK phosphorylation and upregulating FasL and Fas receptor clustering to activate caspase-3 and caspase-8 and then trigger mitochondria-mediated and caspase-dependent pathways in renal carcinoma cells [87]. YC-1 has been shown to downregulate several invasion-related signaling proteins, such as β-catenin, caveolin, Src, and epidermal growth factor receptor (EGFR), as well as multiple growth-related proteins, including 5'-AMP-activated protein kinase α (AMPKα), phospho-acetyl-CoA carboxylase (p-ACC), human epidermal growth factor receptor 2 (HER-2), and mammalian target of rapamycin (mTOR) in nasopharyngeal carcinoma [88]. Other anti-invasion mechanisms of YC-1 have also been identified in nasopharyngeal carcinoma (NPC) cells by reverse phase protein array [88]. Activation of beta-catenin signaling has also been evidenced to involve in inhibiting the proliferation and metastasis of hepatocellular carcinoma using combination therapy with local radiofrequency ablation and YC-1 [89]. Moreover, the previous study indicated that YC-1 has a potential effect to improve drug resistance by inhibiting multidrug-resistant protein resulting in decrease of P-glycoprotein (Pgp) efflux, whose effect is modulated by the NO-cGMP-PKG-ERK signaling pathway [90]. These observations revealed together that YC-1 exerts inhibitory effects in key signaling pathways essential for maintaining cancer or endothelial cell viability and may be developed as an antitumor agent on a broad spectrum of cancer types by facilitating apoptosis and suppressing tumor angiogenesis.

5.3 Neuroprotective and anti-inflammatory effects

The use of NO donors (e.g., NONOate) results in excessive NO production which may cause NO-induced axonal damage by inhibiting mitochondrial respiration, independent of cGMP [91]. YC-1 has been shown to protect white matter axons from NO toxicity. This axonoprotective action of YC-1 was unrelated to its activity on sGC but through a novel action on voltage-dependent Na^+ channels in the rat isolated optic nerve [92]. Lu et al. showed YC-1 inhibition of lipopolysaccharide (LPS)-induced iNOS and cyclooxygenase-2 (COX-2) expression as well as NF-κB activation, implying that YC-1 can be developed as an anti-inflammatory

neuroprotective agent [93]. Chien et al. reported that YC-1 promoted learning behavior in Morris water maze and avoidance tests and YC-1 pretreatment reduced scopolamine-induced learning deficit. Thereby, the NO/cGMP/PKG pathway may be involved in the learning enhancement-based experiments with intracerebroventricular injection of L-NAME and PKG inhibitors [94]. Similarly, YC-1 can also improve age-related learning and memory dysfunction [95]. Furthermore, YC-1 may inhibit HIF-1α accumulation and VEGF production to protect blood-brain barrier against ischemia-/reperfusion-induced injury [96]. In addition, beneficial effect of YC-1 in ameliorating combined allergic rhinitis and asthma syndrome (CARAS) was demonstrated through reducing expressions of HIF-1α, NF-kB, and peroxisome proliferator-activated receptor α (PPARα) [97].

5.4 Optical protections

Therapeutic application of YC-1 on sepsis has been mentioned. After administration with YC-1, several LPS-stimulated modulations, such as NF-κB activation, iNOS expression, NO overproduction, and cytokine release, were markedly inhibited, thus improving survival rate of endotoxemic mice [98]. YC-1 has also been shown to inhibit HIF-1α-induced iNOS and VEGF expressions in various tissue models. Studies showed that YC-1 inhibited optical neovascularization in the pathological stages [99, 100]. Song noted that YC-1 could prevent laser-induced choroidal neovascularization by suppressing photocoagulation-mediated HIF-1 expression [99]. The pathological retinal neovascularization could also be inhibited by YC-1 through decreasing ischemia-induced expression of HIF-1 and its downstream angiogenic mediators (e.g., VEGF) in the ischemic retina. The physiological revascularization of the retinal vascular plexuses was enhanced by YC-1 *via* inhibiting iNOS expression at mRNA and protein levels [100]. Besides, it also has been reported that YC-1 alleviated macular edema in the animal model of laser-induced experimental central retinal vein occlusion by reducing several inflammatory or angiogenesis-related factors, such as interleukin-6 (IL-6), IL-8, VEGF, and HIF-1 [101].

5.5 Other activities

Wang and his colleagues evidenced that YC-1 inhibited bone resorption and induced extrinsic apoptosis of osteoclasts to reduce bone loss, which implied that YC-1 has potential application for use as an antiresorptive drug in postmenopausal osteoporosis [102]. Besides, YC-1 and its derivatives also have been mentioned to improve hepatic fibrosis, which mechanisms may be caused by inhibiting liver neutrophil infiltration as well as decreasing in TNF-α signaling and macrophage aggregation [103, 104].

6. Clinical significance of YC-1

Extensive studies have been conducted to explore possible systemic actions of YC-1 in disease models in animals to demonstrate that YC-1 has versatile physiological activities to be a potent candidate drug for a number of vascular disorders. In the cardiovascular and hematological systems, it has been reported that local extravascular administration of YC-1 could prevent neointima formation in a rat carotid artery model of balloon angioplasty [63, 64, 67]. In a study of experimental thrombosis model, YC-1 conferred beneficial effect through its anti-aggregating and pro-fibrinolytic effects [105]. BAY 63-2521 (riociguat™), a NO-independent but heme-dependent sGC stimulators like TC-1, is currently in clinical development

for the treatment of pulmonary arterial hypertension with the only reported significant side effect to be a decrease in systemic arterial diastolic pressure [106, 107]. Similarly, intravenous administration of YC-1 has been shown to lower mean arterial blood pressure in normotensive and hypertensive rat [108]. For anticancer therapy, Lau et al. demonstrated that intraperitoneal injection of YC-1 enhances cisplatin chemosensitivity of hepatocellular carcinoma cells in nude mice xenograft tumor model, suggesting that YC-1 may be as an adjuvant agent for anticancer therapy [80]. Furthermore, oral administration of YC-1 can also decrease tumor mass in human renal cancer xenograft mice model [87]. In Morris water maze and avoidance test of mice, Chien et al. showed that YC-1 may be a good candidate for the improvement of learning and memory [94, 109]. Hwang et al. demonstrated that YC-1 can potentiate the relaxant responses of exogenous or endogenous NO through the elevation of cGMP in guinea-pig trachea [110]. The above in vivo studies all demonstrated the relevance of YC-1 in association with NO.

7. Conclusions

Accumulating evidences have shown that the administration of YC-1 may have beneficial pharmacological or physiological functions in diseased states for clinical applications. In the future, less toxic and more effective candidates would be the focus of further investigations through structural modification of YC-1 or its derivatives and better understanding of the molecular mechanisms of its actions.

Conflict of interest

The authors state that they have no conflict of interest.

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