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Attenuation of Doxorubicin-induced Cardiotoxicity by Tadalafil: A Long Acting Phosphodiesterase-5 Inhibitor

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Abstract

Doxorubicin (DOX) is a broad spectrum antineoplastic drug widely used in the treatment of several hematogenous and solid human malignancies. Despite its excellent clinical efficacy as a chemotherapeutic agent, its therapeutic usage has been restricted due to its cardiotoxicity. Phosphodiesterase-5 (PDE-5) inhibitors or erectile dysfunction drugs including sildenafil, have been shown to have powerful cardioprotective effect against injuries under a variety of experimental situations including ischemia/reperfusion injury, myocardial infarction and DOX-induced cardiomyopathy. We studied the effect of – tadalafil, a long acting PDE-5 inhibitor in preventing damage in the heart with DOX treatment. Our results showed that tadalafil improved left ventricular function and survival by attenuating DOX-induced apoptosis and cardiac oxidative stress without interfering with the anti-tumor efficacy of DOX in both *in vitro* and *in vivo* tumor models. Herein, we present an overview of our study, and consider the potential mechanisms by which tadalafil, at therapeutically relevant concentrations mediate beneficial cardioprotective effects in DOX cardiotoxicity. Based on our current and previously published studies, we propose that the class of PDE-5 inhibitors can represent a novel approach which can be exploited for achieving therapeutic benefit in the treatment of DOX-induced cardiotoxicity in patients.

Keywords

Doxorubicin; Cardiomyopathy; Phosphodiesterase inhibitors; PKG; cGMP; ROS

Introduction

Doxorubicin (DOX) is an effective antineoplastic drug widely used in the treatment of several hematogenous and solid human malignancies (1,2). However, despite its therapeutic efficacy, its clinical usage is limited by the development of cumulative dose-dependent cardiomyopathy (3) which may occur many years after the cessation of DOX treatment (4,5). The acute DOX-induced cardiotoxicity is characterized by hypotension, arrhythmia, tachycardia while the chronic effects are manifested as cardiac dysfunction eventually leading to congestive heart failure (3,6). The deterioration of cardiac functions is often accompanied with high mortality rates although many patients with ventricular dysfunction may lack overt symptoms until late in their disease.

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Conflicts of Interest
No potential conflicts of interest to disclose.

The exact pathogenesis of DOX-induced cardiotoxicity is still not entirely clear although a diverse set of mechanisms have been proposed, including oxidative stress, mitochondrial DNA damage, intracellular calcium overload, inhibition of protein synthesis, disturbance of myocardial adrenergic function, cytokine release, myofibrillar degeneration and cardiomyocyte apoptosis (7–12). Among the multiple mechanisms, it is widely accepted that DOX-induced cardiomyocyte apoptosis is primarily due to the generation of reactive oxygen species (ROS) in the myocardium which triggers intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes (13). Numerous earlier studies indicate that free radical scavengers and antioxidants may combat DOX-induced cardiotoxicity (14–16), however, despite various therapeutic interventions adapted to protect the heart against DOX-induced cardiotoxicity, most of the antioxidant therapies remained particularly ineffective due to pronounced clinical side effects and due to their inability to specifically target the oxidative damage to cardiac mitochondria (17).

Previous studies from our laboratory have shown that PDE-5 inhibitors like sildenafil (Viagra) and tadalafil (Cialis) which were developed for the treatment of erectile dysfunction in men induced powerful cardioprotective effect during ischemia/reperfusion injury (18–20). Recently, we demonstrated that both sildenafil and tadalafil attenuated cardiac dysfunction in DOX-induced cardiomyopathy (21,22). The pharmacokinetic properties of tadalafil like quick onset of action, longer half-life, relatively slow metabolism, and greater selectivity for PDE-5 enzyme over other PDE isoenzymes and fewer side effects makes tadalafil the preferred choice in the treatment for long-term management of patients receiving DOX for malignant tumors (23). Moreover, the rate and extent of tadalafil absorption is unaffected by food and therefore it could be used at a relatively lower daily dosage during chronic treatment (24). In Koka et al 2010 (22), we have shown that tadalafil has significantly improved the cardiac contractile function which was impaired by DOX with assessment of the left ventricular function by transthoracic echocardiograpghy and millar conductance catheter. In addition, we have also demonstrated, that tadalafil induced the *in vivo* cardioprotective effects and improved survival rates in mice without interfering with the anti-tumor efficacy of DOX by the following mechanisms: 1) attenuating DOX-induced apoptosis, 2) restoring the depleted prosurvival proteins including Bcl-2 and GATA-4, in the myocardium 3) reducing the oxidative stress via the up-regulation of mitochondrial superoxide dismutase (MnSOD) 4) up-regulating of cardiac cGMP and protein kinase G (PKG) activity. In this report, we present an overview on the role of PDE-5 inhibition in protection against DOX-induced cardiotoxicity and further continue to elaborate on the mechanisms by which tadalafil attenuates DOX-induced cardiotoxicity.

Phosphodiesterase-5 inhibitors attenuate DOX cardiotoxicity

Phosophodiesterase-5 (PDE-5) inhibitors are a class of vasoactive drugs that were developed for treatment of erectile dysfunction (ED) in men. The mechanism of action involves active inhibition of the PDE-5A enzyme and resulting increase in cGMP and smooth muscle relaxation in the penis. Sildenafil and tadalafil have also been approved for pulmonary hypertension, but cardiac indications were still considered unlikely given its modest impact on arterial tone and low expression and activity in resting myocytes. This view began to change with the publication of several preclinical landmark studies from our laboratory showing a powerful protective effect of PDE-5 inhibitors including sildenafil (Viagra), Vardenafil (Levitra) and tadalafil (Cialis) against ischemia/reperfusion injury in the rabbit and mouse hearts (18–20). We demonstrated that sildenafil and other PDE-5 inhibitors increase eNOS/iNOS in the heart which leads to downstream protective mechanisms involving cGMP-dependent activation of protein kinase G (PKG) and opening of mitochondrial K_{ATP} channels (25,26). Opening the mitoK_{ATP} channel partially compensates the membrane potential, which enables additional protons to be pumped out to form an H^+

electrochemical gradient for both ATP synthesis and Ca^{2+} transport. The mitochondrial stabilizing effect of sildenafil was further confirmed in our isolated cardiomyocyte study (27), which showed an increase of Bcl-2/Bax ratio and preservation of mitochondrial membrane potential ($\Delta\Psi m$), in the sildenafil-pretreated myocytes. Because DOX-induced cardiotoxicity involvesthe generation of ROS in the mitochondria, we hypothesized thatcardiomyocyte protection by PDE-5 inhibition via opening of mitoK_{ATP} channels, may be extended in demonstrating the prevention of cardiomyocyte apoptosis and subsequent development of cardiomyopathy. Previous studies have shown that the accumulation of ROS results in dissipation of the $\Delta\Psi m$, direct activation of the mitochondrial permeability transition pore (MPTP), and cytochrome c release followed by caspase-3 activation and DNA fragmentation consistent with apoptosis (28).

In a landmark investigation from our laboratory (21), we demonstrated that *in vivo* treatment of mice with sildenafil before DOX administration conferred protective effects in the heart. We observed significant attenuation of cardiomyocyte apoptosis, preservation of myofibrillar integrity, prevention of left ventricular dysfunction and ST prolongation consistent with chronic DOX-induced toxicity 8 weeks after the final of 3 treatments. Sildenafil inhibited DOX-induced $\Delta\Psi m$ dissipation, caspase-3 activation and cardiomyocyte apoptosis (21). Moreover, exposure of adult mouse ventricular myocytes to DOX resulted in dissipation of $\Delta\Psi m$, as illustrated via JC-1 immunofluorescent staining. Moreover, adult cardiomyocytes treated with sildenafil before the treatment with DOX demonstrated preservation of the $\Delta\Psi m$. This protection with sildenafil in DOX-damaged cardiomyocytes was completely abolished by a nitric oxide synthase (NOS) inhibitor (L-NAME) and 5-hydroxydecanoate 5-HD (21). These findings implied that sildenafil-mediated protection from DOX-induced cardiomyocyte apoptosis was NOS dependant and opening of mitoK_{ATP} channel played critical role in attenuation of damage caused by DOX. Additionally, we observed a significant decline in Bcl-2 expression at both 2 weeks and 8 weeks after treatment in the DOX group compared with the sildenafil + DOX and control groups, suggesting a pivotal role of Bcl-2 in altering the pathological process leading to end-stage heart failure.

There is increasing evidence that intermediate filaments such as desmin are involved in cardiomyopathy. Heling et al (29) illustrated the disorganization and accumulation of desmin in explanted human heart specimens from patients with dilated cardiomyopathy. Moreover, desmin-related cardiomyopathy in the knockout ($desmin^{-/-}$) mice was prevented by Bcl-2 overexpression, as evidenced by prevention of cardiomyocyte apoptosis and preservation of cardiac contractility (30). Consistent with findings by Heling et al (29) and Wang et al (31), we observed disruption of desmin distribution in the DOX group compared with the sildenafil/DOX and control groups. Additionally, because desmin is known to adhere to the mitochondria in the same location where the MPTP is formed, it is conceivable that disruption of desmin either through repeated strain on the contractile apparatus resulting from impaired contractility or through direct cleavage from activated caspases may contribute to MPTP formation, cytochrome *c* release, and apoptosis.

In a more recent study, we demonstrated that the long acting PDE-5 inhibitor tadalafil also attenuated cardiac dysfunction in DOX-induced cardiomyopathy (22). Although both PDE-5 inhibitors sildenafil and tadalafil, demonstrated protective effects against DOX-induced cardiomyopathy in mice, tadalafil appears to be the preferred choice for long-term management of patients receiving DOX for malignant tumors owing to its favorable pharmacokinetic properties, relatively slow metabolism, and greater selectivity for PDE-5 enzyme. Moreover, the rate and extent of tadalafil absorption is unaffected by food and hence it could be used at a relatively lower dosage regimen during chronic treatment. In a recent study (22), we demonstrated that tadalafil significantly improved the cardiac

contractile function impaired by DOX treatment. Similar to sildenafil, tadalafil co-treatment with DOX improved survival of mice, restored the depletion of anti-apoptotic protein Bcl-2 and decreased TUNEL-positive apoptotic cells. GATA-4 is a key transcriptional factor which plays a pivotal role in controlling embryonic development, cardiomyocyte differentiation, and stress responsiveness of the heart. It was recently shown that cardiac-specific deletion of GATA-4 resulted in a progressive and dose-dependent deterioration in cardiac function and dilationin adulthood (32). In response to pressure overload, the GATA-4 deficient mice developed rapid decompensation and heart failure. These detrimental phenotypes were associated with increased cardiomyocyte apoptosis (32). We also observed significant down regulation of GATA-4 expression after DOX treatment, which confirmed the previous reports (33), and GATA-4 expression was significantly preserved by co-treatment with tadalafil (22).

Our study also demonstrated that tadalafil treatment inhibited DOX-induced increase in lipid peroxidation. Antioxidant enzymes including Cu/ZnSOD (cytosolic) and MnSOD (mitochondrial) play a critical role in detoxification of ROS. Tadalafil did not affect the regulation of cytoplasmic Cu/ZnSOD but MnSOD was significantly increased. These data imply that mitochondrial elimination of ROS contribute to the cardioprotective effects of tadalafil during DOX-induced toxicity. Previous studies have also shown that MnSOD overexpression can exert cardioprotection against DOX-induced injury and ischemia-reperfusion injury (34). The antioxidant properties of PDE-5 inhibitors have not yet been well understood. Fernandes et al (35) reported that the physiological concentrations of sildenafil (<50 μmol/L) decreased both H_2O_2 generation by mitochondria respiring glutamate/malate. Moreover, it was shown that sildenafil decreased superoxide radical generated by hypoxantine/xantine oxidase system, without affecting either mitochondrial bioenergetics or Ca^{2+}-induced mitochondrial permeability transition (35). Most recently, in a rat model of traumatic spinal cord injury Serarslan et al demonstrated that tadalafil reduced the spinal cord injury via increasing tissue/serum levels of nitric oxide and serum activity of SOD (36). Interestingly, our results demonstrated that co-treatment with tadalafil attenuated ROS generation by DOX, suggesting that tadalafil might have potent antioxidant-like effect, either through upregulation of MnSOD or by direct scavenging of ROS. Based on current state of knowledge, we propose that PDE-5 inhibitors could generate therapeutic levels of NO that result in enhanced formation of cGMP. cGMP may activate PKG that can subsequently open mitoK_{ATP} channels resulting in protective effect against apoptosis by preservation of GATA-4 and Bcl-2, upregulation of MnSOD, attenuating ROS generation, inhibition of MPTP opening in the mitochondria as outlined in Figure 1.

Concluding Remarks

Over the past decade, experimental and clinical studies have reported on the efficacy of PDE-5 inhibition to treat various forms of heart disease including myocardial infarction (25,26), heart failure (37) and hypertrophy (38). These studies have helped in the creation of ongoing NIH multicenter trial (RELAX: Evaluating the Effectiveness of Sildenafil at Improving Health Outcomes and Exercise Ability in People with Diastolic Heart Failure; NCT00763867) in patients with heart failure and a preserved ejection fraction (i.e. EF>50%). Our studies suggest that PDE-5 inhibitors like sildenafil and tadalafil attenuated DOX-induced apoptosis, depletion of pro-survival proteins including Bcl-2 and dissipation of mitochondrial membrane potential ($\Delta\Psi m$) and improved cardiac contractile function which was impaired by DOX. Cardiac oxidative stress was attenuated by tadalafil via upregulation of MnSOD and by direct scavenging of ROS. Moreover, tadalafil treatment increased cardiac cGMP level and PKG activity and restored the depletion of GATA-4 in the DOX treated myocardium. Furthermore, considering the specificity of tadalafil, these studies suggest that PDE-5 is the molecular target for attenuating DOX cardiotoxicity.

Overall, our studies provide valuable new information about the efficacy of tadalafil in attenuation of DOX-induced cardiac dysfunction. Tadalafil exhibits dual mechanisms (Figure 1) i.e. it acts both via mitochondrial antioxidative and cGMP/PKG dependent mechanisms that do not interfere with antineoplastic activity of DOX (22). Currently there is no optimal therapeutic intervention for protecting the heart against the DOX-cytotoxicity. We believe that PDE-5 inhibitors have great potential in the management of the DOX-induced cardiotoxicity and therefore we propose that the class of PDE-5 inhibitors can represent attractive novel therapeutic approach for the treatment of DOX-induced cardiotoxicity in patients.

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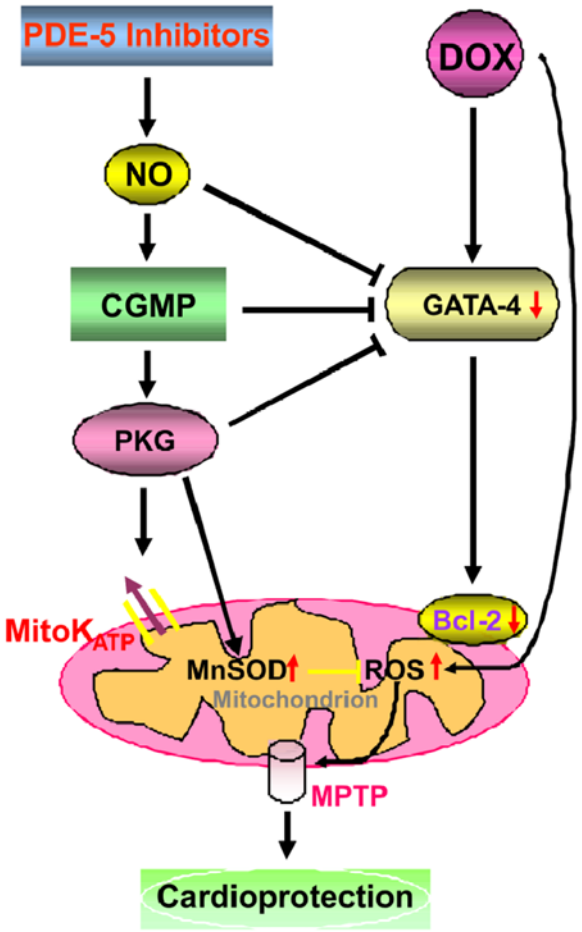

Figure 1. Proposed mechanism of cardioprotection by PDE-5 inhibitors in DOX-induced cardiomyopathy

Abbreviations: ROS- reactive oxygen species, NO- nitric oxide, PKG- protein kinase G, mitoK$_{ATP}$-mitochondrial ATP-sensitive potassium channels, mitochondrial MPTP-mitochondrial permeability transition pore.