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Cardioprotector effect of Phosphodiesterase 5 inhibitors in experimental model for Diabetes Mellitus

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ABSTRACT

Diabetes mellitus (DM) is considered a 21st century pandemic and is often associated with cardiovascular disease (CVD). The aim of this integrative review was to analyze the cardioprotective effects of phosdodiesterase-5 (PDE5i) inhibitors in experimental diabetes models. The articles were selected from the PubMed, SciElo and LILACS databases from 2014 to 2019. The following descriptors were used in combination with the Boolean operators: Diabetes mellitus experimental AND Phosphodiesterase 5 inhibitors; Diabetic cardiomyopathies AND Phosphodiesterase 5 inhibitors. An initial sample of 155 articles was obtained, of which six met the criteria for the synthesis of the review. The studies analyzed showed that treatment with PDE5i in experimental models, resulted in positive effects on cardiac function and metabolic parameters. Similar results have also been seen in humans. The reduction in cardiac hypertrophy, apoptosis of cardiomyocytes, pro-inflammatory factors and oxidative stress and the modulation of transcription factors involved in diabetes homeostasis, were prevalent among studies. The mechanisms of action involved in cardioprotection have not yet been fully elucidated, however the restoration of the activated cyclic guanosine monofate (cGMP) pathway by soluble guanylate cyclase (sGC) via nitric oxide (NO) was a common mechanism among the studies.

Keywords: Diabetes mellitus, experimental; cardiovascular diseases; Phosphodiesterase 5 inhibitors.

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INTRODUCTION

Diabetes mellitus (DM) is among the most prevalent non-communicable chronic diseases in the world population. In 2017, approximately 425 million individuals were affected by DM and this number is estimated to increase to 629 million by the year 2045[1]. Cardiovascular Diseases (CVD), especially Diabetic Cardiomyopathy (DCM), are the leading causes of morbidity and mortality among diabetic patients[2]. DCM is considered a myocardial dysfunction involving epigenetic, molecular, and structural changes, independent of the presence of valvular disease and hypertension[3,4]. It is the main CVD associated with long-term Heart Failure (HF) in diabetic patients, but its current treatment is not well established yet[4,5].

The metabolic changes present in DM such as hyperglycemia, hyperinsulinemia, and increased oxidation of free fatty acids promote increased formation of advanced glycation end products and Reactive Oxygen Species (ROS) with consequent decreased bioavailability of nitric oxide (NO), endothelial dysfunction, and dysregulation of exosomes, which result in mitochondrial dysfunction, oxidative stress, and changes in calcium homeostasis. These abnormalities are associated with cardiac hypertrophy, fibrosis, and myocardial stiffness, leading to diastolic dysfunction and HF[3,6].

In recent years, an interest in the possibility of treating CVD using drugs that act on the signaling pathways of cyclic guanosine monophosphate (cGMP)[7] has emerged. The expression of phosphodiesterase 5 (PDE5), an enzyme responsible for cGMP degradation, is physiologically irrelevant in human heart tissue; however, studies have observed that PDE5 is expressed in the left ventricle (LV) of patients with HF. Thus, the selective inhibition of PDE5 modulates the intensity and duration of the intracellular response of this second messenger[8].

At the cardiac level, cGMP is the product of hydrolysis of guanosine triphosphate (GTP) through the action of two different guanylate cyclases, one located in the plasma membrane (pGC), sensitive to atrial natriuretic peptide (ANP) and the other soluble (sGC), which are NO-activated receptors. The cGMP can be activated directly by cyclic adenosine monophosphate-dependent protein kinase (cAMP) and by cGMP-dependent protein kinase I (cGKI), besides being modulated indirectly by the action of phosphodiesterases (PDE) type 2 and 3[7]. It is worth mentioning that the isoforms PDE5, PDE6, PDE9 present high specificity for cGMP[9]. In this context, experiments that act on the components involved in this signaling pathway, such as the constitutive expression of sGC[10], an overexpression of the ANP receptor, as well as increased expression of cGKI, have demonstrated antihypertrophic and cardioprotective effects[11].

At the vascular level, endothelium-derived NO diffuses into vascular smooth muscle by directly activating sGC, which increase intracellular levels of cGMP by hydrolyzing GTP molecules in a manner similar to that observed in cardiac tissue (Figure 1). In turn, cGMP activates protein kinase G (PKG), a specific cGMP dependent kinase, which phosphorylates several proteins and causes vascular smooth muscle relaxation by reducing intracellular calcium concentrations[12].

The mechanisms of PDE5 inhibitors (PDE5i) are not yet fully elucidated. It is well established that the increase in cGMP levels can result in vasodilation, increased myocardial relaxation and pulmonary vasodilation[13,14].

Thus, it represents a potential pharmacological target, whose main representatives are sildenafil, tadalafil, vardenafil, and avanafil[15].

Considering the therapeutic potential of PDE5i in the treatment of CVDs, the aim of this review was to analyze the results of studies evaluating the cardioprotective effects of PDE5i in models of experimental diabetes, in order to understand the signaling pathways related to possible cardioprotection.

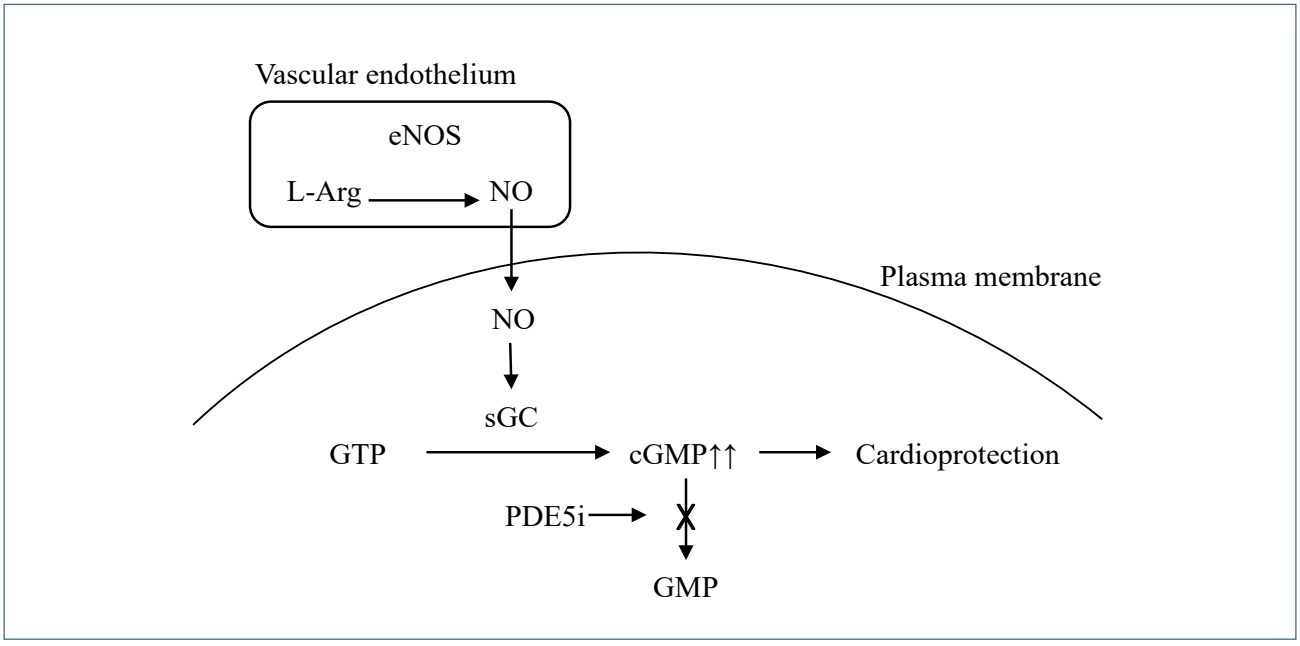


Figure 1: NO-sGC-GMP regulation pathway, potential cardioprotective mechanism of PDE5i. Nitric oxide (NO) is a product of L-arginine conversion by the action of nitric oxide synthase (eNOS). NO activates soluble guanylate cyclase (sGC) which promotes the phosphorylation of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The phosphodiesterase 5 inhibitor (PDE5i) prevents the conversion of cGMP to guanosine monophosphate (GMP). Increased intracellular cGMP levels activate and modulate the intensity and duration of second messengers, promoting cardioprotection.

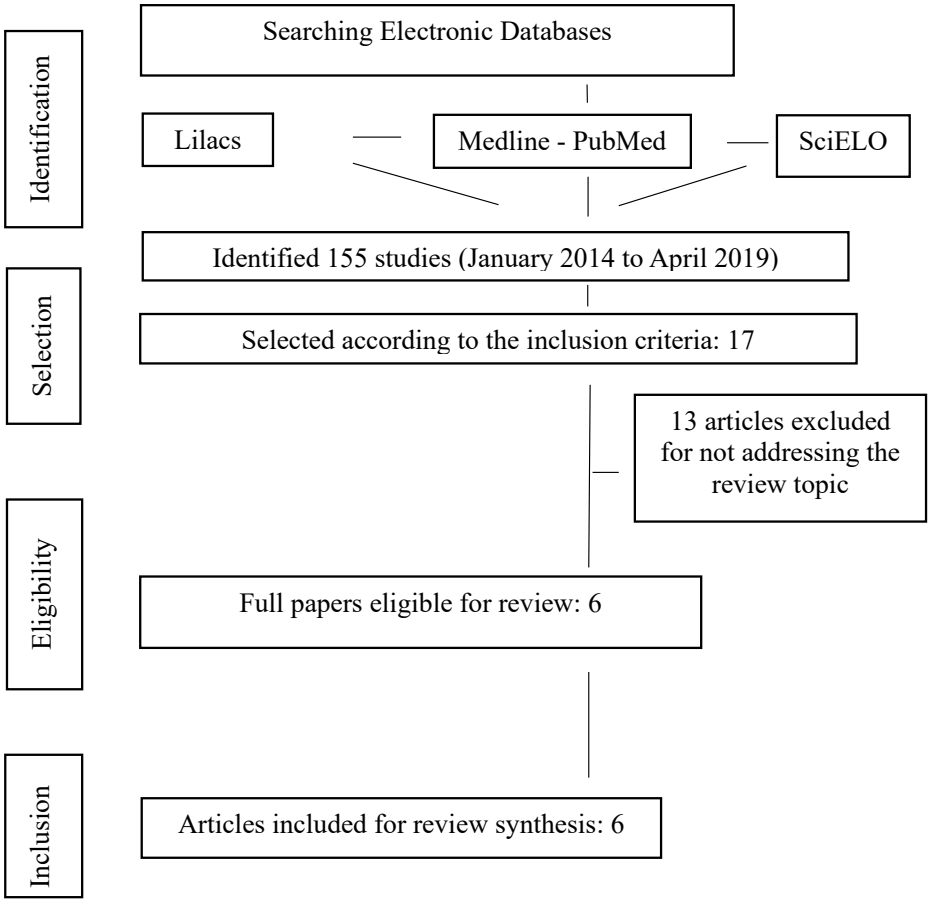
METHODS

This is a qualitative descriptive integrative review that systematically and orderly gathers and synthesizes results from multiple research studies on the effects of PDE5i in the treatment of cardiovascular complications associated with the experimental DM model, as well as answers questions from the theme of interest. This review was divided into six steps: theme identification and selection of research questions; establishment of inclusion and exclusion criteria; identification of preselected and selected studies; categorization of selected studies; analysis and interpretation of results; presentation of the review/knowledge synthesis[16].

The identification of the research theme and elaboration of the guiding question was based on the PICOT strategy[17]. The guiding questions for conducting the review were: would selective inhibition of PDE5 in diabetic rats result in cardioprotection? What are the mechanisms of action involved in cardioprotection after PDE5i treatment in the experimental diabetes model?

The search for articles in order to answer the guiding questions was conducted in the following databases: Medical Literature Analysis and Retrieval System Online (Medline - PubMed), Latin American and Caribbean Literature on Health Sciences (Lilacs), and Scientific Electronic Library Online (SciELO). For this, we used the following descriptors in Health Sciences (DeCS) combined with the Boolean indicator AND: Diabetes mellitus experimental AND phosphodiesterase 5 inhibitors; Diabetic cardiomyopathies AND phosphodiesterase 5 inhibitors, resulting in an initial sample of 155 articles.

To select the studies, the following inclusion criteria were applied: articles available in full, published in Portuguese and English, in the period from January 2014 to April 2019. The following were excluded: abstracts, proceedings of events, monographs, dissertations, theses, and other studies that were not part of the theme of interest. After selection, six full articles were eligible for the review. The search strategy used in the respective databases and the article selection process are presented in the flowchart (Figure 2), which



Lilacs: Latin American and Caribbean Health Sciences Literature, SciELO: Scientific Electronic Library Online, Medline: Medical Literature Analysis and Retrieval System Online (Medline/PubMed).

Figure 2: Flowchart of the article selection process.

followed the recommendations of the systematic review protocol[18]. The characterization of the studies is shown in Table 1.

RESULTS AND DISCUSSION

All published studies that evaluated the potential cardioprotective effect of PDE5i in models of experimental diabetes and correlated the intracellular signaling pathways triggered in these models, in the last 5 years, were of international scope, and most of these were performed in Italy (50%). Regarding the type of study, all were experimental with randomized design with quantitative approach.

Of the multiple animal experimental models, the most frequent was streptozotocin (STZ)-induced diabetes (50%) in adult male Wistar rats and CD1[19-21] mice. Other murine models have also been used, such as leptin receptor deficiency (db^{-}/db^{-})[22], spontaneously obese non-obese diabetic (GK)[23] and spontaneously obese mice (Zucker)[24].

Importantly, among the PDE5i, sildenafil was the most used (66.66%) for the treatment of DM associated with CVD. The treatment with sildenafil was carried out over a period of 3 to 12 weeks at a dose of 1.6 mg/kg and 3mg/kg, respectively[19,20,23]. In other studies, using tadalafil in the db/db model and Vardenafil in Zucker rats, a cardioprotective effect was also suggested[22,24].

The epigenetic studies performed by Bacci et al.[19] and Barbati et al.[20] demonstrated that hyperglycemia reduces NO bioavailability and determines increased transcription of non-coding RNAs (ncRNA) of the class 3 family of histone deacetylases (HDC3), including trimethyl lysine 4 (H3K4mc 3) and lysine 9 acetylation (H3K9ac), compared with nondiabetic animals,

Table 1: Distribution of articles included in the review, according to year, author, country, experimental model and mechanism of action of PDE5i.

Author/Year	Title	Country	Experimental model	Cardioprotective mechanism of PDE5 inhibitors
Bacci et al. 2018[19]	Sildenafil normalizes MALAT1 level in diabetic cardiomyopathy	Italy	STZ-induced diabetes in male Wistar rats	Treatment with sildenafil (3 mg/kg) for 12 weeks normalized the increase in MALAT1 levels in CD1 mouse cardiomyocytes by increasing NO bioavailability, suggesting epigenetic control in the development of CMD.
Barbati et al. 2017[20]	Transcription Factor CREM Mediates High Glucose Response in Cardiomyocytes and in a Male Mouse Model of Prolonged Hyperglycemia	Italy	STZ-induced diabetes in male wistar rats	Exposure to hyperglycemia determined epigenetic variations in cardiomyocytes culture from diabetic rats. Treatment with sildenafil (3mg/kg) for 12 weeks promoted an increase in NO, preventing the nitrolysation of histone deacetylases, which regulate inflammatory factors via cGMP.
Venneri et al. 2015[21]	Chronic Inhibition of PDE5 Limits ProInflammatory Monocyte-Macrophage Polarization in Streptozotocin-Induced Diabetic Mice	Italy	STZ-induced diabetes in non-isogenic mice	Treatment with sildenafil (1.6 mg/kg) for 3 weeks reduced the number of circulating pro-inflammatory monocytes and prevented tissue inflammatory infiltration by reducing iNOS, COX2, VCAM-1), inhibiting fibrosis and cardiac hypertrophy. In diabetic animals sildenafil reduced the mortality rate by 72% within 45 days.
Koka et al. 2014[22]	Chronic inhibition of phosphodiesterase 5 with tadalafil attenuates mitochondrial dysfunction in type 2 diabetic hearts: potential role of NO/SIRT1/ PGC-1 signaling	United States of America	Male db/db mice	Treatment with tadalafil (1 mg/kg) for 8 weeks significantly preserved ejection fraction, attenuated left ventricular dysfunction, and prevented thickening of its wall through phosphorylation of eNOS, via AKT and AMPK, and activation of SIRT1 and PGC1, reducing mitochondrial oxidative stress.
Goulopoulo et al. 2015[23]	Reduced vascular responses to soluble guanylyl cyclase but increased sensitivity to sildenafil in female rats with type 2 diabetes	United States of America	Goto-Kakizaki female mice	Damage to the NO-sGC-GMP pathway was demonstrated through impairment of endothelium-dependent and endothelium-independent relaxation to acetylcholine to stimulators/agonists when compared to AMR from control rats. The increased sensitivity to PDE5i confirmed a cardioprotective effect of sildenafil.
Matyas, et al. 2017[24]	Prevention of the development of heart failure with preserved ejection fraction by the phosphodiesterase 5A inhibitor vardenafil in rats with type 2 diabetes	Hungary	Male Zucker Rats	The pre-treatment with vardenafil (10mg/kg) for 25 weeks promoted cardioprotection, via regulation of cGMP levels. In treated animals there was a reduction in cardiac hypertrophy, oxidative stress, fibrosis and apoptosis levels in cardiomyocytes, preventing left ventricular dysfunction when compared to the control group.

Diabetic Cardiomyopathy (DCM); Cyclooxygenase 2 (COX-2); Peroxisome proliferator-activated receptor α alpha coactivator 1 (PGC-1) Streptozocin (STZ); Phosphodiesterase 5 (PDE5); Soluble guanylate cyclase (sGC); Cyclic guanosine monophosphate (cGMP); Phosphodiesterase 5 inhibitor (PDE5i); Cyclic adenosine monophosphate response element modulator (CREM); Vascular cell adhesion molecules-1 VCAM-1); Obesity induced leptin receptor deficiency (db/db); Nitric oxide (NO); Inducible nitric oxide (iNOS); Nitric oxide synthase (eNOS); Sirtuin 1 (SIRT1); Adenosine monophosphate-activated protein kinase (AMPK); Protein serine kinase (AKT); Metastasis-associated lung adenocarcinoma transcript-1 (MALAT1).

destabilizing the function of metabolic and epigenetically active enzymes. Prolonged hyperglycemia is a determining condition for increased EROS and reduced NO bioavailability, leading to vascular impairment and cardiac dysfunction. Thus, treatment with sildenafil prevented changes in epigenetic programming in the experimental diabetes model induced by STZ and in cardiomyocyte cultures incubated with high glucose concentration (30 mM)[19,20].

The study by Lu et al.[8] also correlated increased cardiac oxidative stress to elevated PDE5 levels. Tadalafil treatment in diabetic mice exerted an anti-inflammatory effect, which was related to a reduction in the area of the cardiac infarction, a reduction in postprandial glucose and triglyceride levels, as well as in tumor necrosis factor-alpha and interleukin 1β levels. Attenuation of apoptosis and tissue necrosis also occurred[25]. Chronic treatment with PDE5i was also able to suppress EROS production, oxidized glutathione levels, and lipid peroxidation[26,27].

It has been shown that there is a relationship between diabetes and disturbances in NO levels, but it is not yet fully established how this change occurs. Sildenafil was a PDE5 inhibitor used in several studies; the results indicate that it was effective in promoting cGMP accumulation, as well as modulating the effects of NO, the β-adrenergic response, and markers associated with tissue damage, fibrosis, and cardiac remodeling[24], pigenetic programming points to new therapeutic strategies in the treatment of CMD[19,20,22].

In the study by Koka et al.[22], an important action of tadalafil was evidenced in the regulation of sirtuin 1 protein 1 (SIRT-1) expression, a silent information regulator, which controls the energy status and contractile function of the myocardium, via transcription factor coactivator (PGC-1), in the control of mitochondrial biogenesis and oxidative metabolism. NO also plays an important role in SIRT-1 activation and expression, and dysregulation of both is associated with diabetes-related CVDs. It is important to highlight that in CMD mitochondrial dysfunction is present with consequent increased oxidative stress, drugs that act by reducing lipid oxidation and restoring mitochondrial function are of great interest for the improvement of this condition. SIRT-1 represents a therapeutic target in the control of a number of metabolic disorders, including DM and obesity, since it promotes anti-inflammatory effects, improves insulin sensitivity, and increases mitochondrial biogenesis. In this study, tadalafil increased plasma NO levels and the protein expression of SIRT-1 and PGC-1 in the myocardium, via phosphorylation of the endothelial NO synthase enzyme[22].

In recent years, experimental studies have shown that the presence of comorbidities, especially obesity and DM 2, lead to increased EROS, decreased NO bioavailability and reduced levels of cGMP and PKG[26,27]. Treatment for seven weeks in Zucker mice (fa/fa) with Vardenafil (10 mg/kg) prevented diastolic dysfunction in this model, since it reversed the levels of cGMP, PKG activation, in addition to reducing myocardial apoptosis and cardiac hypertrophy[22].

In a study of vascular reactivity in resistance mesenteric arteries (RMA) from GK mice, an impairment of the NO-cGMP pathway was observed, demonstrated by impaired endothelium-dependent and endothelium-independent relaxation to acetylcholine, NO-independent but heme-dependent stimulators of sGC, as well as direct agonists of sGC, independent of the heme site of sGC and NO, when compared with RMA from control rats. However, an increased sensitivity to PDE5i confirms a cardioprotective effect of sildenafil, which shows promise in initiating vascular dysfunction of diabetes in GK rats[23].

It is reported in the literature a biological action of PDE5i in the modulation of macrophages in STZ-induced DM with reducing effects on inflammation and tissue remodeling[22,27]. They are associated with reduced levels of circulating proinflammatory cytokines and with cardioprotection, but these anti-inflammatory effects are not yet well established. Chronic pro-inflammatory M1-type macrophage infiltration supports endothelial dysfunction and insulin resistance, correlating DM with CVDs. Hyperglycemia and oxidative damage trigger endothelial dysfunction, pro-inflammatory and pro-thrombotic state. In this context, M1-type macrophage infiltration occupies a prominent role[22].

Endothelial cells are also important for the release of inflammatory mediators and adhesion molecules, including vascular cell adhesion molecule (VCAM), an important membrane protein and marker of endothelial dysfunction. Treatment with sildenafil increased the expression of M2-type macrophages, as opposed to the proinflammatory condition with M1-type macrophages, and also reduced VCAM expression in diabetic rats, decreasing monocyte adhesion, increasing the survival of these animals[21]. Moreover, studies have reported that diabetes increased the expression of Cyclooxygenase-2 (COX-2), however, treatment with PDE5i reduced the expression of both, suggesting a new mechanism of PDE5i in modulating inflammation contributing to the cardioprotective effect[21,22].

As in experimental models, a randomized clinical trial in humans observed that LV hypertrophy and altered myocardial contractile dynamics are early findings of cardiac dysfunction in diabetes. In addition, PDE5 expression and reduced cGMP levels promoted cardiac remodeling. Treatment of diabetic patients with sildenafil 100 mg/day for 3 months demonstrated a significant reduction in LV mass, improved cardiac relaxation and remodeling. The regulation of growth factor (TGF-β) and serum monocyte chemoattractant protein-1 was also observed in the clinical study28. In contrast to the experimental studies, no significant differences were found in blood glucose, insulin or lipid profile after treatment.

In experimental models, can be considered limiting factors for defining the cardioprotective mechanism of PDE5i the use of the Zucker obese rat (fa/fa) model of insulin resistance and obesity, the difficulty in measuring cGMP production in

resistance arteries in response to activation of sGC, as well as the duration of diabetes, the type of treatment, blood glucose levels, and oxidative stress, which differ according to the stage of the disease.

Conclusion

The results showed that PDE5 inhibitors have a potential cardioprotective effect in experimental diabetes models, and positive results were also found in humans. PDE5i were able to modulate transcription factors involved in glucose and insulin homeostasis, reduce inflammatory mediators and oxidative stress, prevent apoptosis of cardiomyocytes, and reduce LV dysfunction. Cardioprotection may be provided by different metabolic pathways, which are not yet fully elucidated, but regulation of the NO-sCG-cGMP axis presents itself as a common pathway among most of the studies analyzed.

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