Original Article

Effect of sildenafil-induced nitric oxide on the histomorphology of cardiomyocytes in male rats

Latha V Prabhu, Rajalakshmi Rai, Anu V Ranade[1], Hema Kini[2], Ashwin Krishnamurthy, Kavitha Leigelin Bernhardt[3]

Departments of Anatomy, [2]Pathology, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India, [1]Department of Anatomy, Gulf Medical University, Ajman, United Arab Emirates, [3]Department of Physiology, Kasturba Medical College International Center, Manipal University, Manipal, Karnataka, India

Abstract

Introduction: Although sildenafil citrate, one of the selective phosphodiesterase-5 (PDE5) inhibitors, is considered the best treatment for erectile dysfunction, studies have shown that it has also a beneficial effect on a variety of cardiovascular conditions. In spite of reports of a significant protective effect of sildenafil against necrosis in intact hearts, there are also contradictory reports regarding its beneficial effect on the heart. Since there are not enough reports regarding the histomorphological changes in the cardiomyocytes after exposure to sildenafil citrate, the present study was conducted to observe the same along with other biochemical parameters. **Materials and Methods:** Adult male albino rats of Wistar strain were used in the present study. The animals were divided into a control group and two experimental groups containing six rats each. The animals were treated with a solution of sildenafil citrate dissolved in distilled water. Histomorphological changes were observed by light microscopy and the levels of nitric oxide (NO) and PDE in the heart were measured by spectrophotometry. **Results:** It was observed that animals treated with sildenafil citrate showed a highly significant increase in NO and a decrease in PDE level, but the histological architecture of the cardiomyocytes did not show much change other than a slightly elongated and swollen nucleus. **Conclusions:** This study shows that sildenafil citrate at low dosage is well tolerated by cardiac muscle cells, but as dosage increases, it may become detrimental through its NO and PDE activity.

Key words: Cardiomyocytes, erectile dysfunction, nitric oxide, phosphodiesterase-5 inhibitor, sildenafil

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Introduction

Among all phosphodiesterase-5 (PDE5) inhibitors sildenafil citrate is considered one of the prize-winning because of its efficacy[1-4] and pleiotropic effects.[5] Also in recent years, there have been several studies on sildenafil for its therapeutic applications in diseases other than erectile dysfunction (ED).[6-12] Kumar *et al*.,[13] stated that patients with heart failure and preserved ejection fraction might derive particular benefit from these drugs. However, unfortunately, as the availability of sildenafil has become easier, it is being used and misused by many.[14] Reports in letters to the Editor of the *British Medical Journal* state that sildenafil is used as a recreational drug in younger generations.[15] On account of the increasing incidences of sudden cardiac deathsamong ED patients treated with sildenafil citrate, it becomes essential to understand how this drug affects the heart. Therefore the present study was conducted to find out the histological changes in the cardiomyocytes and correlate it with the biochemical parameters.

Address for correspondence: Dr. Rajalakshmi Rai, Department of Anatomy, Kasturba Medical College, Bejai, Mangalore - 576 104, Karnataka, India.
E-mail: rajalakshmi.rai@manipal.edu.

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Materials and Methods

Adult (14-16 weeks old) male albino rats of Wistar strain were used in the present study. The animals were divided

into two groups. Group-I (control group (n = 6)) rats were intraperitoneally injected distilled water for the duration of the experiment on alternate days. Group-Ia and Ib (experimental groups (n = 6 in each group)) rats were intraperitoneally injected 10 mg/kg body weight (bw) and 8 mg/kg bw of sildenafil citrate, respectively, for 30 days, on every alternate day at a regular time interval. The dosing solution was prepared by grinding Viagra tablets into powder and dissolving in distilled water (concentration: 3.5 mg/ml of water).[16,17] On the last day of the treatment, rats were anaesthetized with pentobarbital sodium (45 mg/kg bw) 1 h after injecting sildenafil citrate. The experimental protocol was approved by the Institutional Animal Ethics Committee of Manipal University (Vide No.: 245/2005). The heart was removed from the killed rats and part of the left ventricle was used for histopathological analysis and the other part for biochemical investigations.

The part of the tissue for histological analysis was fixed in 10% formalin for 18 h. Following fixation, the specimens were dehydrated, embedded and then sectioned to 5 μm thickness. For histological examinations, sections were stained with Ehrlich hematoxylin and eosin.[18] Multiple slides were examined for each group: At least three slides from different areas of the tissue were examined. Each tissue was examined with a standard light microscope.

Tissue for biochemical estimation was homogenated in 10 ml of phosphate-buffered saline (pH 7.4). The homogenate was tested for nitric oxide (NO) and PDE. NO was estimated by Griess reagent method[19] and PDEwas measured by spectrophotometry.[20,21]

Data analysis

Results are expressed as means ± standard deviation (SD). Kruskal–Wallis test was performed to measure any differences between the mean values of the different groups. If a difference was found, groups were compared using Wilcoxon signed rank sum test. Probability value (P-value) less than 0.05 was considered significant.

Results

Results of biochemical analysis

NO level

Animals in Group-Ia showed a highly significant ($P < 0.05$) increase and those in Group-Ib showed a significant ($P < 0.01$) increase in the level of NO in the heart when compared with their control group (Group-I) [Table 1].

PDE level

There was a highly significant decrease ($P < 0.01$) in the level of PDE in the heart of animals in Group-Ia in comparison with the control group, whereas changes in PDE level in Group-Ib animals were not significant when compared with those in the control group animals [Table 1].

Results of histological analysis of heart

Histological evaluation of heart sections from animals of Group-Ia showed a few morphological changes in their microstructure. The nucleus of the muscle fibers in this group was slightly swollen and elongated. In Group-Ib animals the cardiac muscles did not show any morphological changes in their microstructure when compared with the control group animals.

Discussion

Although studies of intact hearts have demonstrated a significant protective effect of sildenafil against necrosis (infarction), there is absolutely no information in the literature regarding the histological changes in cardiomyocytes. After an early case report suggesting that PDE5A inhibitors might increase the risk of heart attack,[22] several studies attemptedto define the cardiac effects of this class of drugs. Results of direct analysis of cardiac effects, which have been obtained *in vitro*, remain limited and conflicting. It is said that PDE1 is abundant in ventricular myocytes and sildenafil shows far less affinity for other PDE isozymes, including PDE1.[23] There has been a dominant view that PDE5 is not present in the myocardium.[23,24] Cheitlin *et al*.,[24] stated that PDE5 is not present in cardiomyocytes, instead PDE3 is found and sildenafil is comparatively less potent on this isoenzyme.[24] The present study shows that changes in the level of total PDE were statistically significant at a dosage of 10 mg/kg bw when compared with the 8-mg/kg bw and the control groups. This suggests that as the dosage of sildenafil increases it becomes more potent on PDE in heart, indicating the presence of PDE5. This is in agreement with the results of a few previous studies, which showed that PDE5 is found in specific compartments within myocytes (specifically at z-bands).[17,25] Cremers *et al*.,[26] found no effect of 10 μM sildenafil (a relatively high dose) on isoprenaline-stimulated function in human papillary muscle strips. PDE5 inhibition has also been shown to suppress and reverse pressure over-load-induced ventricular hypertrophy,[27] attenuate apoptosis[17] and reduce post-ischemic dysfunction[28] in mice, which appear to be critically coupled to the NO synthase. In the present study, at 8 mg/kg bw cardiomyocytes did not show any histological changes, indicating the negligible effect of sildenafil citrate on the heart at a low dosage.

Table 1: NO level in heart in the control and experimental groups

	Control-I	Group-Ia	Group-Ib
Nitric oxide (OD)	49.67±2.34	101.80±6.23	54.39±1.013
PDE (OD)	7.92±0.32	6.64±0.19	8.15±0.18

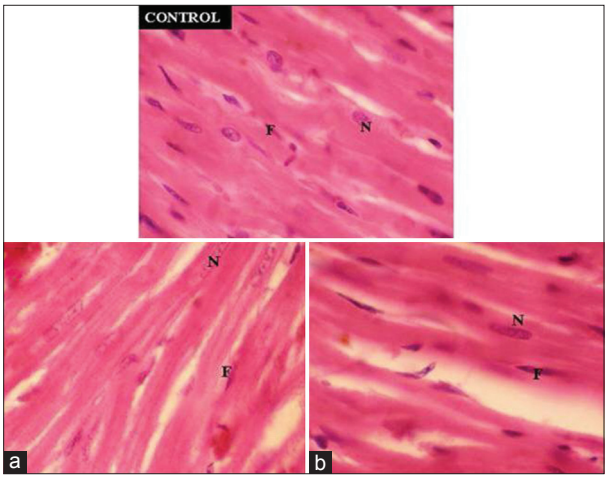

Figure 1: Photomicrographs of cardiac muscle tissue. Magnification: (H and E, ×40). Control: Animals treated with distilled water. (a) Group-Ia (10 mg/kg bw sildenafil citrate). (b) Group-Ib (8 mg/kg bw sildenafil citrate); N: Nucleus of cardiomyocytes; F: Fibrocytes

However, as dosage increased (10 mg/kg bw) the nucleus of the cardiac muscle became swollen and elongated. Tracqui *et al*.,[29] have described the death of a 56-year-old man associated from an overdose of sildenafil citrate. Histological examination of the ventricular myocardium revealed some areas of patchy fibrosis and moderate hypertrophy of the myocytes.

Fisher *et al*.,[28] hypothesized that the vasodilatory action of sildenafil could potentially release endogenous mediators of preconditioning such as adenosine or bradykinin from endothelial cells, triggering the phosphorylation of NO synthase (NOS) and subsequent release of NO. If the increased production of NO is well in balance with a moderate increase in oxygen radicals then NO will exert beneficial effects.[30] In the present study, although there was significantly high ($P < 0.05$) level of NO when compared with the control group at a high dosage (10mg/kg bw), histological differences were fewerthan the control group animals [Figure 1]. Therefore it can be concluded that sildenafil citrate at a low dosage does not appear to influence cardiomyocytes. However, NO-induced changes may become detrimental to the cardiac tissue as the dosage of the sildenafil increases.

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