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The Effect of Low Dose Sildenafil on Verapamil–Induced Cardiovascular Toxicity in Rats

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Abstract

Background: Experimental studies have shown that sildenafil may have significant cardio protective effects if used in low doses. The aim of this study was to compare the efficacies of glucagon and sildenafil treatments in anesthetized rats receiving verapamil overdose.

Methods: Male Sprague-Dawley rats (n = 8 per group), weighing 300-350 g were used in the study. The iliac arteries and veins of rats bilaterally were catheterized under urethane anaesthesia. Toxicity was induced by infusion of verapamil 15 mg/kg until 10 minutes. The rats received continuous infusion of sildenafil (0.06 mg/kg/h), glucagon (2mg/kg/h) or 0.3mcg/kg/min noradrenaline added to sildenafil 0.06 mg/kg/h infusion.

Results: The AUC10-60 min of MAP plot was significantly lower in the control group, and both treatments produced significant increases in the AUC10-60 min of MAP plots ($p < 0.05$). Noradrenaline added to the sildenafil infusion was found to generate better values in the cardiovascular parameters ($p < 0.01$). Heart rate values were restored better in the group that received noradrenaline adjunct.

Conclusion: The results of this study may imply that sildenafil alone is no better than glucagon, the classical treatment approach in verapamil toxicity. However, sildenafil may be more effective if used with a vasopressor adjunct.

Keywords: Sildenafil; Verapamil; Intoxication; Cardioprotection; Cgmp;

Introduction

Calcium-Channel Blockers (CCBs) one of the most important classes of cardiovascular drugs and overdose associated with significant morbidity and fatal outcomes. CCBs exert their toxic effects by interfering with calcium cell influx blocking "L" type voltage-dependent channels [1]. Poison-Induced Cardiogenic Shock (PICS) potentially lethal inhibition of calcium entry and results conduction abnormalities in the sinoatrial and atrioventricular nodes.

Beyond general supportive care, the goals of therapies for the management of CCA drug toxicity are to achieve improved perfusion by increasing blood pressure and reversing myocardial dysfunction.

Glucagon is an effective antidote for drug-induced myocardial failure with the direct myocardial action in both ischemic and nonischemic heart failure. Glucagon's positive chronotropic and inotropic pharmacokinetic effects on the myocardium are well characterised with therapeutic activity and increased cardio dynamic changes [2-4]. Several animal studies and case reports have also demonstrated a benefit in CCB toxicity, though many treatment failures have been noted as well.

Sildenafil, a selective inhibitor of phosphodiesterase-5A (PDE-5A) enzyme that hydrolyzes cGMP, increases the concentrations of cGMP in the cortex, hippocampus, and striatum, was originally developed to treat angina pectoris and subsequently has been approved for erectile dysfunction, it now has other medical indications such as pulmonary hypertension, spinal cord injury and type II diabetes [5,6]. Furthermore, it has been shown that administration of sildenafil may also be useful against ischemia and reperfusion (I/R) injury [7,8].

In the present study, we designed this experiment to evaluate the cardioprotective effect of low dose of sildenafil in a rat model of lethal verapamil overdose which aims to PDE-5A inhibition, and cellular accumulation of cGMP would be the braking force against drug-induced cardio toxicity.

Methods

Chemicals and Reagents

Sildenafil citrate and urethane were supplied by Sigma-Aldrich (St. Louis, MO, USA) and Glucagon by Novo Nordisk (A/S, Basvaerd, Denmark).

Animals and Experimental Design

The study design is by the local ethical comity of Marmara University School of Medicine, Animal Research Committee (Approval number: 53.2015.mar). Handling and care of the animals were by European guidelines for ethical animal research. Forty male Sprague-Dawley rats (n=8 per group), weighing 300-350 g were used in the study. The animals were housed in individual cages, at constant temperature conditions (21ºC),

with alternating 12-hour light/dark cycles. They were also maintained on a standard diet and water ad libitum. Experiments were performed under urethane (Sigma, St. Louis, MO, USA; 1.2 g/kg, intraperitoneal) anaesthesia. Normal body temperature was maintained by a heating pad and body temperature was continuously monitored via a rectal thermometer and during the experiments.

Direct measurement of blood pressure

The iliac artery and vein of rats were catheterized under urethane anaesthesia. Upon completion of catheterization, the rats were connected to a monitör (BeneView T5, Shenzhen Mindray Bio-Medical Electronics Co) to observe blood pressure and heart rate throughout the procedures. All catheters were filled with 1% heparin (Liquemine. Roche, Istanbul, Turkey) in physiological saline solution.

Experimental protocol

The animals were randomly allocated into five groups: a group which received no treatment(control group, = 8), a toxicity group which was induced by infusion of verapamil 15 mg/kg until 10 minutes (group II, = 8), a group that received continuous infusion of sildenafil (0.05 mg/kg/h) after toxication (group III, = 8), another group that received continuous infusion of glucagon (2mg/kg/h) after toxication(group IV, = 8), and a group that received 0.3mcg/kg/min noradrenaline added to sildenafil 0.05 mg/kg/h infusion (group V, = 8). The rats were sacrificed by cervical dislocation at 60 min.

Myocardial cGMP Analysis

The GMP levels in the heart were measured using an ELISA kit according to the manufacturer's instructions (ADI-900-014, Enzo Life Sciences, USA). Briefly, the µg of protein lysates from the isolated hearts and standards were added to the cGMP conjugated to alkaline phosphatase and an anti-cGMP antibody and incubated for 2 hours at room temperature. This incubation allows the antibody to bind the cGMP in the sample or conjugate in a competitive manner. After 3 washes, p-nitrophenyl phosphate (pNpp) substrates were added and the plated and incubated for 1 hour at room temperature. This incubation allows the catalysis of the pNpp substrate by the alkaline phosphatase on the cGMP conjugate. The reaction was then stopped by tri-sodium phosphate solution, and the optical density was read at 405 nm. The amount of signal is indirectly proportional to the amount of cGMP in the sample.

Statistical Analysis

Data were analysed using Graph pad Prism 4R (GraphPad Software, San Diego, CA). The level of statistical significance was set to < 0.05. To make an integrative comparison, the area under the curve in the pressure vs. time plot of each rat was calculated. One-way analysis of variance was used followed by Tukey's test for statistical analysis. The results were expressed as mean ± SEM. The MAP was calculated as 1/3 pulse pressure + diastolic blood pressure.

Results

Baseline age, weight, heart rate, respiratory rate, and rectal temperature were the same for the control and treatment groups. All the animals manifested signs of cardiovascular toxicity within minutes of commencement of the verapamil infusion. The hemodynamic data for all three groups at baseline and the point of toxicity are shown in figure 1-2. All the rats in groups survived to the completion of the 90-minute observation period.

Verapamil infusion produced decreases in the Mean Arterial Pressure values (MAP). The AUC(10-60 min)of MAP plot was significantly lower in the control group, and upon completion of the verapamil infusion the systolic blood pressure immediately started to increase for the rats that treated with glucagon and sildenafil and both treatments produced significant increases in the AUC(10-60 min) of MAP plots ($p < 0.05$). Noradrenaline added to the sildenafil infusion was found to generate better values in the cardiovascular parameters ($p < 0.01$). Comparison of pulse pressure did not yield a significant difference. Heart rate values were restored better in the group that received noradrenaline adjunct.

As shown in Figure 3, sildenafil didn't alter a significant increase in myocardial cGMP formation compared to untreated controls ($P > 0.05$).

Discussion

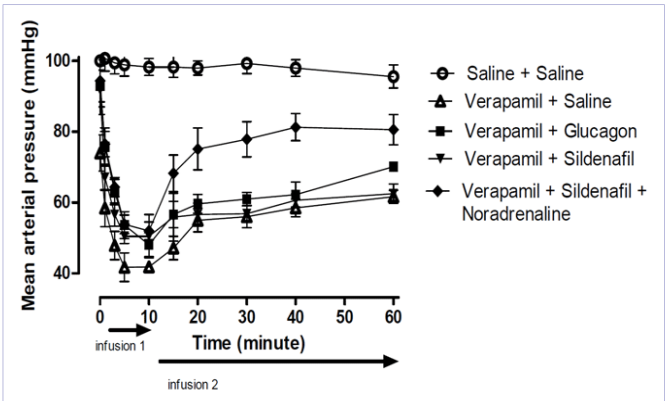

Figure 1: The MAP(mean ± SD) of rats during verapamil intoxication with treatment groups. Infusion 1 indicates infusion of verapamil for toxicity; infusion 2 shows different treatment groups after drug overdosage.

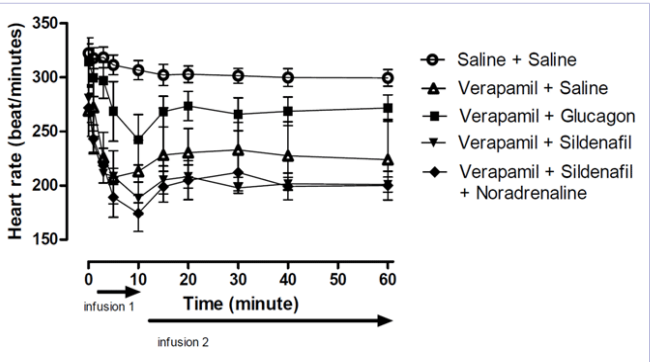

Figure 2: Heart rate (HR) of rats during the procedure. Infusion 1 indicates infusion of verapamil for toxicity; infusion 2 shows different treatment groups after drug overdosage.

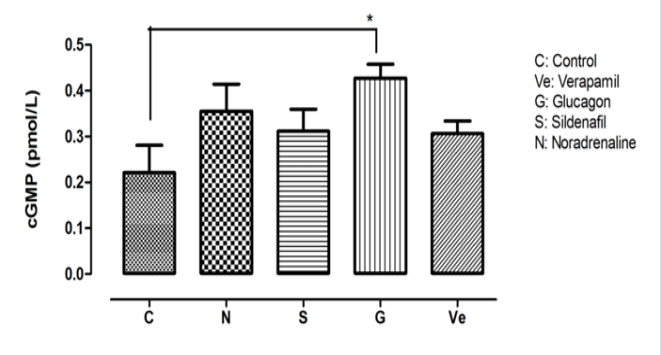

Figure 3: The comparison of GMP levels in the heart according to groups which are measured by using ELISA.

To our knowledge, sildenafil has not been used experimentally or clinically to reverse acute drug-induced cardiac failure. This current experimental study for a rat model of verapamil overdose using sildenafil or glucagon treatment increased MAP, compared with the control group, but did not improve heart rate or pulse pressure to the same degree as did sildenafil + noradrenaline therapy. While the sildenafil or glucagon alone increases systemic arterial pressure, it appears to be less effective than sildenafil+noradrenaline for reversal of the hemodynamic effects of experimental verapamil overdose. An interesting observation in the present study was that absolute level of cGMP was not altered by sildenafil after verapamil poisoning. As sildenafil improves the hemodynamic parameters in low doses, the results of the present study may indicate that sildenafil-induced cardioprotection is not dependent on myocardial cGMP levels.

Over the past years, enormous progress has been made in understanding the mechanisms underlying myocardial injury by calcium channel blockers poisoning and has led to the development of several experimental cardioprotective strategies. However, only a few of them have been integrated into clinical pathways yet. Verapamil and diltiazem poisoning related shock is the result of cardiac toxicity and vasodilation so that treatment might require a combination of inotropic, chronotropic and vasoconstrictor agents.

Recent studies have reported the protective effects of phosphodiesterase type 5 inhibition in various models of cardiac pathology. Recently it was shown that sildenafil has a powerful acute cardioprotective effect by reducing ischemia-reperfusion injury of the isolated perfused rat heart. The results of this study implicated that sildenafil-induced acute cardio protection was completely abolished by a calcium channel blocker before sildenafil injection and suggested that opening of mitochondrial calcium channels is essential for the early cardioprotective effect of sildenafil with enhanced cGMP synthesis. Also, it was proposed that the acute cardioprotective effect of sildenafil occurs independently from potential vasodilating effects in the coronary vasculature [9]. In a model of isoproterenol intoxication, which uses the same pathway with CCBs, sildenafil use in a chronic manner was found effective against ventricular hypertrophy and myocardial cell injury by increasing the cellular cGMP levels [10].

In contrast to the previous studies, it was demonstrated that treatment with low dose sildenafil (0.06 mg/kg) 5 min before ischemia-reperfusion injury significantly reduced myocardial infarct size that is independent of myocardial cGMP levels. Results of this study may suggest that acute low dose sildenafil-mediated cardioprotection is independent of eNOS, iNOS, and cGMP [11].

The inhibiting cAMP breakdown may be effective in CCB overdoses with improved heart rate and systemic blood pressure by a positive inotropic effect [12]. Phosphodiesterase Inhibitors (PDEs) like amrinone and milrinone directly stimulate myocardial contractility independent of adrenergic receptors and exacerbates hypotension because of vasodilatation. These agents should be used in concurrently with a vasopressor since when used alone they may likely cause peripheral vasodilatation, and this may counteract the inotropic effects on blood pressure [13]. In human case reports, PDEs showed the increase of inotropy with a lesser vasopressor requirement [14,15]. Unfortunately, the use of amrinone did not improve inotropy or with little effect on heart rate in animal studies. PDEs currently hasn't been recommended as a routine therapy for CCB intoxication [16-19]. Due to the limited experience and unclear optimal dosing regimens with PDEIs they should be considered as third-line options in poisoning when other vasoactive agents have failed [20,21].

Conclusion

The present study represents the first report showing that sildenafil improves hemodynamic parameters after verapamil poisoning and no integrity of the cGMP signalling pathway plays a pivotal role in the cardio protection. Further clinical study of sildenafil in verapamil poisoning should be done to determine a more efficient treatment regimen for this drug.

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Declarations:

The authors have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Disclaimer:

The view expressed in this paper represents the views of the authors and is not an official position of the institution

Ethical Approval:

The study design is by the local ethical comity of Animal Research Committee (Approval number: 53.2015.mar).

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