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# Effects of Sildenafil a Phosphodiesterase 5 Inhibitor on Rat Liver Cell Key Enzymes of Gluconeogenesis and Glycogenolysis

Sheyda Hoseini, Hadi Esmaily, Azadeh Mohammadirad and Mohammad Abdollahi
Laboratory of Toxicology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre,
Tehran University of Medical Sciences, Tehran, Iran

**Abstract:** In the present study, the effects of short-term administration of sildenafil a selective PDE5 inhibitor on serum glucose and hepatic glycogenolysis and gluconeogenesis were examined in rats *in vivo*. Sildenafil was administered intraperitoneally at doses of 0.5, 1.0 and 5.0 mg $kg^{-1}$. Two hours post treatment, liver was perfused, removed and homogenized. The activities of the key enzymes of glycogen phosphorylase (GP) and phosphoenolpyruvate carboxykinase (PEPCK) were analyzed in the homogenate. The high dose of sildenafil (5 mg $kg^{-1}$) caused a significant reduction in serum glucose levels in comparison to control group (94.6±4.7 vs. 121.16±6.05 mg $dL^{-1}$). The activity of PEPCK remained unchanged when animals were treated with various doses of sildenafil. Interestingly, sildenafil reduced hepatic GP at all doses administered (20.31±1.42, 17.18±1.2, 15.69±1.1 vs. 25.8±1.8 U $g^{-1}$ liver protein for control group). It is concluded that administration of sildenafil markedly reduces liver glycogenolysis which in turn lowers blood glucose concentration at higher doses. This effect of sildenafil seems to be in relation with its NO mimicking potential and antioxidant properties. The underlying mechanisms involved seem complicated since parallel increase in the activities of GP and PEPCK was not observed.

**Key words:** Liver, gluconeogenesis, glycogenolysis, cAMP, cGMP

## INTRODUCTION

The phosphodiesterases phosphodiesterase (PDEs) are a superfamily of enzymes which catalyze the hydrolysis of the cyclic nucleotides cAMP, cGMP to their corresponding inactive 5-monophosphate counterparts. The cyclic nucleotides play a prominent role in the regulation of cellular functions and PDE inhibition can therefore elicit a variety of effects (Dal-Piaz and Giovannoni, 2000). There are 11 families of PDE enzymes (PDE1-PDE11) which are recognized by substrate specificity, kinetic characteristics and amino acid sequence. Sildenafil is a potent and selective inhibitor of PDE5 that decreases the breakdown of endogenously produced cGMP in smooth muscle cells and nerves (Ballard *et al*., 1998) and is currently used for treatment of male erectile dysfunction (Souness *et al*., 2000). The pharmacologic actions of phosphodiesterase inhibitors (PDEIs) are due to their potential to increase intracellular cAMP and cGMP as intracellular signal messengers that modulate both the intensity and the nature of immediate and delayed cellular responses (Soderling and Beavo, 2000).

The recent studies in animals (Milani *et al*., 2005) and human (Rahimi *et al*., 2005; Aydin *et al*., 2001) have demonstrated an impact of PDE5 inhibitors upon glucose metabolism but the reports are controversial. In some studies, cGMP accumulation in the liver cells has been shown to have little effect on glycogenolysis (Atefi *et al*., 2004; Pointer *et al*., 1976) while on the other hand, the inhibition of hepatocyte protein synthesis and gluconeogenesis caused by cytokines and nitric oxide (NO) has been attributed to an increase in cGMP (Parker *et al*., 1997) level. Also, T-1032, a new cGMP PDE-5 inhibitor has shown potent inhibitory influence on the action of physiologic insulin in capillary recruitment and glucose uptake (Mahajan *et al*., 2003). There is evidence that liver is one of the organs that well responds to PDE inhibitors by influencing glucose output (Abdollahi *et al*., 2003a). The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. If the liver is overloaded with glucose, a hepatic steatosis can develop together with high glycogen content. Conversely, if glucose availability in the diet is reduced, glucose-utilizing pathways are inhibited and glucose-producing pathways are activated. During fasting, glucose is produced by the liver glycogenolysis. Then, when the glycogen stores are depleted, glucose is produced de novo from precursors such as lactate, alanine or fructose through a pathway called

**Corresponding Author:** Mohammad Abdollahi, Laboratory of Toxicology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Tehran 14155-6451, Iran Tel: 98 21 66959104

gluconeogenesis. The regulation of these metabolic pathways involves the rapid modulation of the activity of specific proteins (enzymes, transporters) and modulating their transcription rate or post-transcriptional steps such as mRNA half-life and translation efficiency (Abdollahi *et al*., 2003b; Foufelle and Ferre, 2002; Nordlie *et al*., 1999). Regarding above-mentioned reports, the aim of this study was to examine whether effect of administration of sildenafil (selective PDE5 inhibitor) might affect activities of hepatic key enzymes of gluconeogenesis and glycogenolysis including phosphoenolpyruvate carboxykinase (PEPCK) and glycogen phosphorylase (GP) in rat.

## MATERIALS AND METHODS

**Materials:** All chemicals were obtained from Sigma-Aldrich (Dorset, England).

**Animals:** Adult male Wistar rats, supplied by the animal house of Faculty of Pharmacy, Tehran University of Medical Sciences, weighing 200-250 g were used in this study. All animals were housed under standard laboratory conditions and fed normal laboratory rat chow and water.

**Treatment:** The animals were housed under controlled environmental conditions of temperature (22±2°C) and a 12 h light/dark cycle. Animals were allowed to acclimatize for 7 days. They were fed with standard rat chow and had free access to food and water. The protocol of study was approved by TUMS/PSRC ethic committee.

**Sampling:** Sildenafil was administered intraperitoneally at doses of 0.5, 1.0 and 5.0 mg $kg^{-1}$. Two hours later, the liver was removed under anesthesia by transverse abdominal incision and perfusion with cold 0.9% saline. The weight of liver was recorded and kept frozen at -70°C until homogenized. The activities of the key enzymes of phosphoenolpyruvate carboxykinase (PEPCK) and glycogen phosphorylase (GP) were determined in liver homogenate to identify changes in glycogenolysis and gluconeogenesis respectively, as described previously (Saadat *et al*., 2004; Abdollahi *et al*., 2004a). Blood sample was taken under anesthesia by cardiac puncture and serum was separated and kept at -20°C for glucose assay.

**Glucose assay:** Serum glucose level was measured in the presence of glucose oxidase and peroxidase using o-dianisidine-HCl as a chromogen. The amount of glucose formed is related to amount of o-dianisidine oxidation products that measured spectrophotometrically at 436 nm (Bergmeyer *et al*., 1974).

**Determination of PEPCK activity in liver:** The liver homogenate was mixed with 10 volumes of cold 0.2 M phosphate buffer, pH 7.4, containing 2 mM mercaptoethanol and 2 nM EDTA and was then mixed and centrifuged for 10 min at 60000×g at 0°C and the supernatant was used for enzyme activity assay. Enzymatic activity was assayed in the reverse direction, carboxylation of phosphoenolpyruvate to form oxaloacetic acid in the presence of NADH as previously described (Chang and Lane, 1966). PEPCK activity is expressed as unit per gram of liver protein.

**Determination of GP activity in liver:** The liver homogenate was centrifuged at 30000×g for 30 min and the supernatant was used for enzyme activity assay. Enzymatic activity was assayed in the direction of glycogen breakdown by measuring the reduction of NADP (Lowry *et al*., 1967). GP activity is expressed as unit per gram of liver protein.

**Determination of liver protein concentration:** The liver homogenate was centrifuged as described above and the total protein concentration was measured in the supernatant using bovine serum albumin as standard by Lowry procedure (Lowry *et al*., 1951).

**Statistical analysis:** The results were analyzed for statistical significance by one-way ANOVA test and Tukey's post hoc multi-comparison. All data were expressed as means±SEM of six animals in each group. A $p<0.05$ was considered statistically significant.

## RESULTS

Administration of sildenafil at doses of 0.5 and 1.0 mg $kg^{-1}$ did not alter serum glucose concentration but the dose of 5.0 mg $kg^{-1}$ caused a significant reduction (22.41%, $p<0.01$) in comparison to the control group (94.6±4.7 vs. 121.16±6.05 mg $dL^{-1}$, Fig. 1). Administration

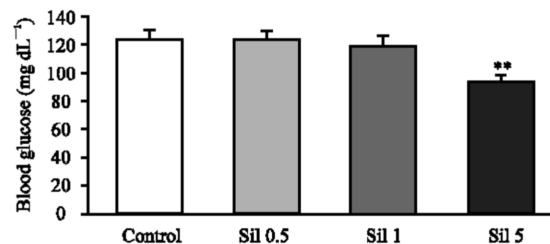


Fig. 1: Effects of sildenafil on rat blood glucose concentration. Sildenafil (Sil) was administered in different doses of 0.5, 1 and 5 mg $kg^{-1}$. Values are expressed as mean±SE of 6 animals in each group. ** Significantly different from control at $p<0.01$

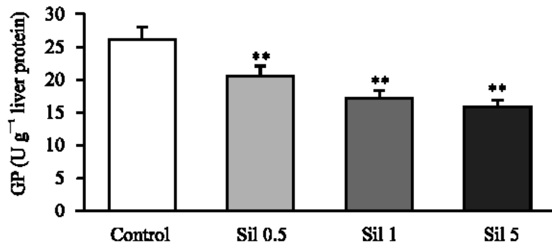


Fig 2. Effects of sildenafil on rat hepatic glycogen phosphorylase (GP) activity. Sildenafil (Sil) was administered in different doses of 0.5, 1 and 5 mg kg$^{-1}$. Values are expressed as mean±SE of 6 animals in each group. ** Significantly different from control at p<0.01

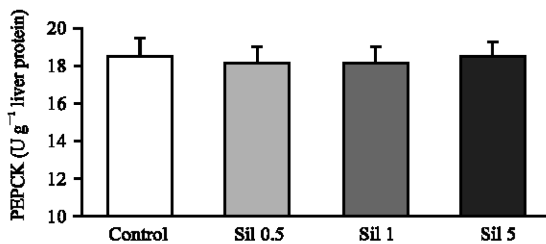


Fig. 3. Effects of sildenafil on rat hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity. Sildenafil (Sil) was administered in different doses of 0.5, 1 and 5 mg kg$^{-1}$. Values are expressed as mean±SE of 6 animals in each group

of sildenafil at doses of 0.5, 1.0 and 5.0 mg kg$^{-1}$ reduced the hepatic GP activity in comparison to control group (20.31±1.42, 17.18±1.2, 15.69±1.1 vs. 25.8±1.8 U g$^{-1}$ liver protein, Fig. 2) with a mean percentage of decreases of 21, 33 and 39%, respectively. The activity of PEPCK was not changed after administration of sildenafil (Fig. 3).

## DISCUSSION

The results of this study indicated that *in vivo* treatment of rats by sildenafil dose-dependently reduces hepatic GP activity without affecting PEPCK. Interestingly, only high dose of sildenafil affected blood glucose and reduced its level. GP is the key enzyme of glycogen breakdown that catalyzes the degradative phosphorolysis of glycogen to glucose-1-phosphate. This enzyme is promoted by substrate as well as allosteric effectors, including adenosine monophosphate (AMP) and phosphorylation (Barford and Johnson, 1989). There is evidence that glucagon and other cAMP-increasing agents exert a glycogenolytic effect by maintaining GP in a phosphorylated active state (Bollen *et al.*, 1998). cAMP or $Ca^{2+}$ dependent glycogenolytic agents cause glycogen degradation through specific protein kinases which may activate the GP. Considering the ability of sildenafil to reduce hepatic GP activity, one mechanism would be that sildenafil inhibits cAMP-dependent processes. To explain this, it should be noted that increasing cGMP is a fundamental mechanism of action of sildenafil. Our recent study on rat hepatocytes *in vitro* indicated that sildenafil increases hepatocytes cGMP levels but does not significantly alter glycogenolysis and gluconeogenesis (Atefi *et al.*, 2004; Abdollahi *et al.*, 2003c; Abdollahi and Simaiee, 2003). That is partly in agreement with the present findings when considering that blood glucose does not significantly change with normal doses of sildenafil. Sildenafil, through the inhibition of PDE5 and augmentation on the action of NO-cGMP axis shows similar effects to NO in many organs. Recently, NO has been suggested as a second messenger molecule for the stimulatory effect of insulin in carbohydrate metabolism (Khan *et al.*, 2000; Khan and Sinha, 1990). Incubation of various tissues, including heart, liver, kidney, muscle and intestine from mice and erythrocytes or their membrane fractions from humans, with physiologic concentration of insulin results in the activation of a membrane-bound NOS and synthesis of NO. Furthermore, NO has shown an insulin-like effect in stimulating glucose transport and glucose oxidation in muscle, a major site for insulin action that is completely blocked in the presence of NOS inhibitor. Also, injection of NO in alloxan-induced diabetic mice mimics the effect of insulin in the control of hyperglycemia by lowering plasma glucose concentration (Kahn *et al.*, 2000; Czech, 1990; Khan and Sinha, 1990). There is also evidence that a combination of tumor necrosis factor a, interferon γ and interleukin 1β plus lipopolysaccharide in hepatic cultures result in an induction of NOS and concomitant inhibition of hepatic gluconeogenesis and glycogenolysis (Ceppi and Titheradge, 1998; Casado *et al.*, 1996). Similarly, the hypoglycemia that is seen in septic shock, is the result of reduced liver glucose output while the peripheral glucose utilisation is enhanced (Ceppi *et al.*, 1989). In addition, it has been reported that culturing hepatic parenchymal cells in the presence of insulin results in an NO-dependent inhibition of the glucagon-stimulated rate of glucose production via gluconeogenesis plus glycogenolysis (Stadler *et al.*, 1995). NO can inhibit both the basal and the glucagon-and cyclic AMP-mediated mobilization of glycogen (Ceppi and Titheradge, 1998). Also NO is shown to stimulate glycolysis through a glycogenolysis-independent mechanism and a concomitant increase of glycogen possibly through inhibition of glycogenolysis (Cidad *et al.*, 2004). These mentioned mechanisms all

explain the inhibitory effect of sildenafil on glycogenolysis and also its blood glucose lowering effect.

On the other hand, a role for reactive oxygen intermediates in the regulation of hepatic glucose production has been reported (Ceppi and Titheradge, 1998). Troglitazone inhibits expression of the PEPCK gene in isolated hepatocytes by an antioxidant property due to existence of the alpha-tocopherol (vitamin E) moiety in its chemical structure (Davies *et al*., 2001). The antidiabetic drug metformin which acts through inhibition of hepatic gluconeogenesis produces concurrent antioxidant effect that is beneficial in the treatment of diabetes (Cosic *et al*., 2001). Recent studies indicated a protective role for cGMP from induction of oxidative stress inside cells (Aghababaeian *et al*., 2005; Radfar *et al*., 2005; Astaneie *et al*., 2005; Abdollahi *et al*., 2004b; Jahanshahi *et al*., 2004; Abdollahi *et al*., 2003c). It has been reported that sildenafil, through the inhibition of PDE5 and augmentation of inhibitory action of the NO-cGMP axis on NADPH oxidase expression and activity inhibits superoxide formation in corpus cavernosal smooth muscle cells (Koupparis *et al*., 2005; Shukla *et al*., 2005). Thus antioxidant potential of sildenafil might be another mechanism for its glucose lowering effect and inhibition of glycogenolysis.

Taken collectively, it is concluded that sildenafil reduces glycogenolysis which in turn lowers blood glucose concentration especially at higher doses. This effect of sildenafil seems to be consistene with its NO mimicking potential and antioxidant properties. Additional studies are required to determine whether the same effects could be seen in humans.

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