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Research Paper

Sildenafil and Human Platelet Aggregation

S. K. Verma* and Preetesh Jain

Department of Medicine and Indigenous Drug Research Centre
RNT Medical College, Udaipur, Rajasthan, India

***Corresponding Author Email:** drskverma77@gmail.com

ABSTRACT

The effect of the phosphodiesterase-5 inhibitor, sildenafil, on platelet aggregation ex vivo was observed in 30 healthy volunteers. Sildenafil, either 50 or 100 mg, was randomly given to the selected study subjects, and platelet aggregation was observed initially and 2 and 4 hours after sildenafil administration. Adenosine diphosphate (ADP) and collagen were employed as agonists to induce aggregation. Sildenafil, in a single dose of 100 mg, significantly inhibited collagen-induced platelet aggregation at 2 hours ($p<0.05$) and 4 hours ($p<0.001$). Fifty mg sildenafil did inhibit platelet aggregation induced by collagen at 2 and 4 hours after its administration but significantly only at 4 hours ($p<0.05$). ADP induced platelet aggregation, however, it was not significantly inhibited by sildenafil in either dose.

KEYWORDS: Phosphodiesterase, Platelet aggregation, Sildenafil

INTRODUCTION

Sildenafil has been studied and found useful in erectile dysfunction in men with a variety of underlying disorders[1,2] and in women with selective serotonin reuptake inhibitors (SSRIs)-associated sexual dysfunction[3]. Studies in animals and other pharmacodynamic studies have explored its use in esophageal motility dysfunction, [4]lung fibrosis, and pulmonary hypertension[5].

Sildenafil acts by inhibiting cyclic guanosine monophosphate (cGMP), specifically, phosphodiesterase-type-5 (PDE 5) enzyme, which is mainly located in vascular smooth muscle cells and platelets. Physiologically, nitric oxide (NO), released on stimulation, activates the enzyme guanylate cyclase. This, in turn, increases levels of cGMP, which causes smooth muscle relaxation. In the penis, this allows an inflow of blood leading to erection. The cGMP in turn is degraded by PDE 5. Once PDE 5 is inhibited by a phosphodiesterase inhibitor such as sildenafil, the effects of cGMP are enhanced[6,7].

Human platelets have been reported to contain 3 isomers of phosphodiesterases (types I, III, and V).[8] Studies *in vitro* have shown that sildenafil is selective for PDE 5. Its effects are more potent on PDE 5 than on other known phosphodiesterases. It is tenfold more potent than PDE 6. eightyfold more potent than PDE 1, and more than a thousand-fold more potent than PDE 2, 3, and 4. The approximately 4000-fold selectivity for PDE 5 vs. PDE 3 is important because PDE 3 is involved in control of cardiac contractility[9].

The activation of human platelets can be inhibited by 2 intracellular pathways regulated by either cGMP or cyclic adenosive monophosphate (cAMP). However, nitric oxide causes the activation of cGMP-dependent protein kinases, which prevents the agonist-induced activation of

myosinlight chain kinase and protein kinase-C and inhibits the agonist-induced calcium mobilization from intracellular stores without any major effect on the ADP-regulated cation channel. Additionally, cGMP causes an increase of cAMP by inhibition of cAMP phosphodiesterases. Increased cGMP 10 levels inhibit agonist-induced platelet aggregation[10]. Dipyridamole, an ADP uptake and PDE 5 inhibitor, has been extensively used as an antithrombotic agent in clinical application[11]. Moreover, there are reports that PDE 5 inhibitors inhibit platelet aggregation in animal models and that sildenafil exerts antithrombotic effects in combination with a nitric oxide donor in a rat model[12-14]. These findings indicate that inhibition of PDE 5 may influence platelet aggregation. There are few reports that show in-vitro data of human platelet modulation by the PDE 5 inhibitor sildenafil[15-16]. The present study has, therefore, been envisaged to investigate further whether sildenafil in different doses alters human platelet aggregation induced by ADP and collagen.

Methods

The present study was approved by the local ethics committee and conducted on 30 healthy male volunteers between the ages of 30 and 50 years. Underlying diseases like diabetes, hypertension, ischemic heart disease (IHD), hyperlipidemia, kidney diseases, and liver diseases were excluded by relevant investigations. The volunteers were not consuming tobacco in any form and had not taken any type of drug in the previous 15 days. After informed consent, the study subjects were randomly divided into 2 groups of 15 each. Group I (n=15) was administered sildenafil 50 mg and Group II (n=15) was administered sildenafil 100 mg in a single oral dose.

Platelet Aggregation

After an overnight fast 4.5 mL of venous blood was collected without undue pressure and a single dose of sildenafil (50 or 100 mg) was administered. Subsequent blood samples were collected 2 and 4 hours after drug administration. All blood samples collected were mixed to 3.8% of 0.5 ml. of sodium citrate in a 9:1 blood-to-anticoagulant ratio in plastic tubes and subjected to the estimation of platelet aggregation. Platelet-rich plasma (PRP) was prepared by centrifugation of anticoagulant sample at 250 × g for 10 minutes at room temperature. Aliquots of PRP (450 µL) were placed in disposable polystyrene cuvettes.

Platelet-poor plasma (PPP) was obtained by re-centrification of the original blood sample at 1,500 × g for 10 minutes. Platelet aggregation was measured turbidimetrically on ELVI-840 aggregometer and Omniscribe chart recorder. The measurement of aggregation was performed exactly after 30 minutes of sample collection to avoid differences of the aggregation due to altered status of the platelet resulting from ex-vivo conditions. After 10 minutes of equilibrium at 37°C and constant stirring at 1,000 rpm, the aggregation of PRP was induced by ADP (6 µmol/L) or collagen (0.2 µg/mL) (Sigma), the response was recorded for 5 minutes, and the results were expressed as percentage aggregation[17].

Statistical Analysis

All data are expressed as mean $\pm$ SE. The results were analyzed with Student's t test for paired data. A p value of <0.05 was considered statistically significant.

Results

Platelet aggregation was always measured exactly 30 minutes after blood sampling to avoid differences of the aggregation due to altered status of the platelets resulting from ex-vivo conditions. ADP (2 x 10-4mol/L)-induced platelet aggregation in both dose schedules (50 and 100 mg) was not significantly inhibited at 2 and 4 hours after sildenafil administration (Tables 1, II Figure 1).

Table 1: Effect of 50 mg Sildenafil on Platelet Aggregation in Healthy Individuals (n = 15)

	Initial	Platelet Aggregation (%) 2 Hours	4 Hours
ADP	59.73 $\pm$ 3.66	61.16 $\pm$ 2.50	55.390 $\pm$2.34
p Value		NS	NS
Collagen	54.02 $\pm$ 4.58	50.20 $\pm$ 3.94	43.75 $\pm$ 4.34
p Value		NS	< 0.05

p Value as compared to initial, NS = Not significant

Table 2: Effect of 100 mg Sildenafil on Platelet Aggregation in Healthy Individuals (n = 15)

	Initial	Platelet Aggregation (%) 2 Hours	4 Hours
ADP p Value	56.67 + 2.58	56.25 +2.27 NS	55.03+2.84 NS
Collagen p Value	52.92 + 4.05	42.17 + 4.90 < 0.05	29.67 + 5.09 < 0.001

p Value as compared to initial, NS = Not significant

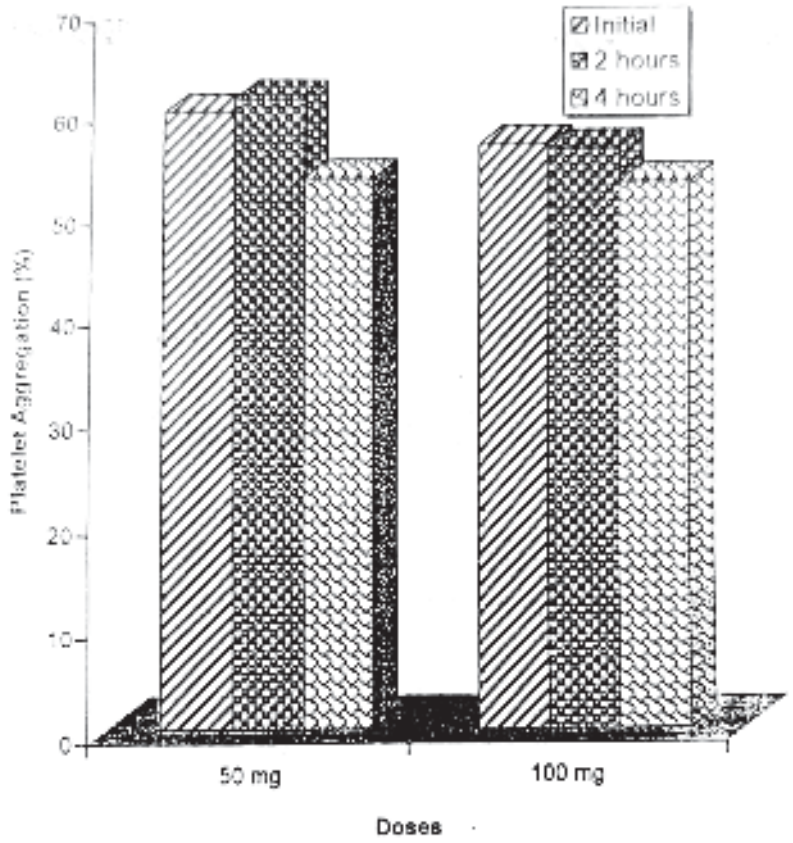
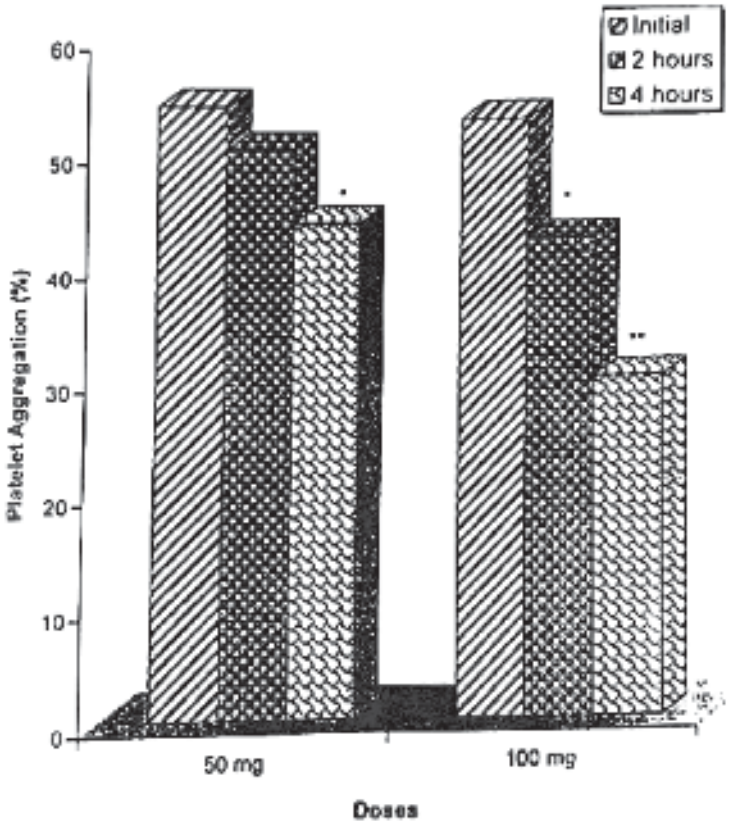


Figure 1: No Significant inhibition of ADP-induced platelet aggregation by Sildenafil

Sildenafil, 100 mg, significantly decreased collagen (0.2 µg/mL)-induced platelet aggregation after 2 ($p<0.05$) and 4 hours ($p<0.001$) (Table II, Figure 2). This effect was, however, overcome when larger concentrations of collagen (>0.2 µg/mL) were used to induce the aggregation (data not shown). This is probably due to the almost maximal aggregation (>70%) achieved with the larger concentration. When 50 mg sildenafil was administered, the collagen-induced platelet aggregation was inhibited at 2 and 4 hours but was statistically significant only at 4 hours (Table 1, Figure 2).



*** p< 0.05**
**** p< 0.001**

Figure 2: Significant inhibition of collagen-induced platelet aggregation by Sildenafil

Side Effects

Sildenafil was well tolerated. Incidences of side effects such as flushing, heaviness of head, stuffiness of nose, and blue vision were higher in those who received 100 mg of sildenafil. The side effects declined, however, after 2 to 3 hours.

DISCUSSION

Time and experience have established the role of sildenafil in the treatment of erectile dysfunction of varied etiologies[2,7]. It is a selective inhibitor of cyclic guanosine monophosphate (cGMP), specifically the phosphodiesterase 5 (PDE 5) enzyme, which is mainly located in vascular smooth muscle cells and platelets. As the cyclic nucleotides are important inhibitors of platelet activation 10 and aggregation, there were investigated in the present study to determine whether medication with sildenafil in healthy volunteers alters platelet aggregation *ex vivo*.

In the present study inhibition of collagen-induced platelet aggregation by sildenafil was clearly evident. The response was dose dependent. Administration of 50 mg sildenafil significantly ($p<0.05$) inhibited collagen-induced platelet aggregation at 4 hours but not at 2 hours. On increasing the dose to 100 mg the platelet aggregation inhibitory response was also significant at 2 hours ($p<0.05$) and was highly significant ($p<0.001$) at 4 hours. Moreover, in 3 volunteers there was complete inhibition of collagen-induced platelet aggregation. As the changes in aggregation are best seen during use of submaximal aggregation stimuli, we used collagen in a concentration of 0.2 µg/ml. to achieve platelet aggregation around 50-55% and not higher concentrations, which can lead to maximal aggregation (>70%), and the effect may be overcome. ADP-induced platelet aggregation, on the other hand, was not significantly inhibited by either dose.

Human platelets have been reported to contain 3 isomers of phosphodiesterase (types I, III, and V)[8]. The activation of human platelets can be inhibited by 2 intracellular pathways

regulated by either cGMP or CAMP. However, nitric oxide causes the activation of cGMP-dependent protein kinases. Additionally, cGMP causes an increase in cAMP by inhibition of cAMP phosphodiesterases[18]. Increased cGMP levels inhibit agonist-induced platelet aggregation.

There are also reports that PDE 5 inhibitor inhibited platelet aggregation and adhesion in animal models[14,15] and that sildenafil exerted antithrombotic effect in combination with a nitric oxide donor in a rat model[12]. There is 1 report that shows in vitro data on human platelets, which were incubated with sildenafil or sodium nitroprusside, or both[15].

ADP and collagen act on platelets via different receptors[19,20], although the underlying signal transduction is not fully understood. However, it is difficult to relate the inhibitory effect of sildenafil on collagen-induced aggregation to a specific pathway in the signal cascade, Unlike ADP, it is possible that only collagen triggers an endogenous nitric oxide release of platelets during activation or aggregation[21,22].

The nitric oxide/cGMP metabolism in platelets is probably activated during aggregation to prevent exceeding aggregation and to act as a negative feedback mechanism. Until now only collagen could be shown to activate this mechanism of generating cGMP, which is degraded by phosphodiesterase type 5[21,22]. This might explain why *in vitro*, without a nitric oxide source such as endothelium, an ADP-induced aggregation is not inhibited by pre-treatment with sildenafil, in contrast to a collagen-induced aggregation.

The above-cited hypothesis is well substantiated by a few of the studies, in which addition of nitric oxide donor before aggregation significantly inhibited ADP and collagen-induced aggregation after administration of the PDE 5 inhibitor sildenafil[16] or zaprinast[23] in doses that otherwise do not inhibit aggregation.

The present observation is important in view of sildenafil administration to subjects receiving other antiplatelet or anticoagulant medications. Sildenafil might further enhance the anti aggregating response and cause bleeding. A recent study[16] has already reported that it enhances bleeding time in volunteers who received sildenafil. However, there are no published data on whether the bleeding time of patients treated with anticoagulants (coumarin) or anti-platelets (aspirin/clopidogrel) is influenced by sildenafil.

Moreover, the positive approach of the present observation is that the drug may prove to be beneficial in those who have incipient endothelial dysfunction, which is now a common denominator in erectile dysfunction, and in the patient with cardiovascular disease. New frontiers about the uses of sildenafil now center around its capability of enhancement of nitric oxide, a signaling molecule that controls a diverse range of physiological processes in many tissues and plays a role in the immune system, nervous system, and in inflammation[24]. The present observation is a further addition to its therapeutic potential.

CONCLUSION

The present study indicates that sildenafil inhibits collagen-induced platelet aggregation *ex vivo*. The effect is greater with a 100 mg dose than with a 50 mg dose of sildenafil; however, ADP-induced platelet aggregation was unaffected by both dose schedules. The exact mechanism is not very clear. However, it is possible that collagen triggers an endogenous nitric oxide release of platelets during activation or aggregation and sildenafil enhances the effect of nitric oxide, a signaling molecule.

CONFLICT OF INTEREST: None

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