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Letter to the Editor

On the interference of sildenafil on platelet aggregation: An ex vivo pilot study

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Doping

Dear Editors,

Sildenafil citrate is a potent vasodilating agent mainly used for erectile dysfunction [1]. In the recent years, its use has also become more popular among men without erectile dysfunction to enhance sexual performance [2]. In addition, although not included in the WADA (World Anti-Doping Agency) prohibited list, the increasing incidence of its use among young athletes has drawn the attention of the anti-doping authorities, who recognized its potential use associated with steroid doping (http://www.realchampion.jp/assets/uploads/2013/03/2010_ProhibitedList).

The drug is a selective inhibitor of phosphodiesterase type 5 (PDE-5) that increases the action of nitric oxide by preventing the hydrolysis of cyclic guanosine monophosphate. PDE-5 is located not only in the corpus cavernosum (the main target of this drug) but also, in other cells, including vessel cells and circulating cells [3].

Accordingly, evidence from clinical trials and experimental studies suggested that sildenafil could exert a plethora of pharmacological beneficial actions in different clinical matters [4]. For instance, in patients with pulmonary arterial hypertension sildenafil improves pulmonary exercise capacity and quality of life by increasing the supply of blood to the lungs. Furthermore, it has been shown that sildenafil has a potential therapeutic efficacy for disorders related to the central nervous system. It exerts neuro-protective effects in multiple sclerosis and has a significant memory enhancing action [4]. However, unfortunately, some adverse effect of the drug has also been reported. Acute myocardial infarction, sudden cardiac death, symptomatic non-fatal ventricular arrhythmia, transient cerebral ischemic attacks, and hemorrhagic stroke have been documented in patients engaging in sexual exertion assisted with the use of sildenafil [5,6].

Since platelet hyperactivity may cause thrombotic disorders [7] whereas their hypo-aggregation can lead to hemorrhagic disorders [8] in the present work we decided to evaluate the ex vivo effects of sildenafil on platelet aggregation pattern in a small cohort of young and healthy individuals (HD). Twenty non-smokers HD men, aged between 20 and 40 years have been recruited at the Institute of Hematology, University "La Sapienza" of Rome. The nature and purpose of the study were explained to all participants who gave their informed consent following the rules of good medical practice.

Whole blood from HD, collected in tubes containing citrate, was treated for 15 min with sildenafil (Sigma) at concentrations of 20, 40 and 80 μM [9]. Platelet aggregation was evaluated in the whole blood by using a Multiplate aggregometer and induced by adenosine diphosphate (ADP, 6.5 μM) and collagen (3.2 μg/ml). The main advantage of whole blood aggregometry is that the evaluation of platelet function can be performed under near physiological conditions, where platelets can interact with other blood cells. For each donor the measurements are performed in duplicate. A value of $p < 0.05$ was considered as significant.

As shown in Fig. 1, we found that sildenafil reduced platelet aggregation induced by both ADP (Fig. 1A) and collagen (Fig. 1B) in a dose-dependent manner. ADP is an important physiological platelet agonist released from erythrocytes and platelets. Under physiological

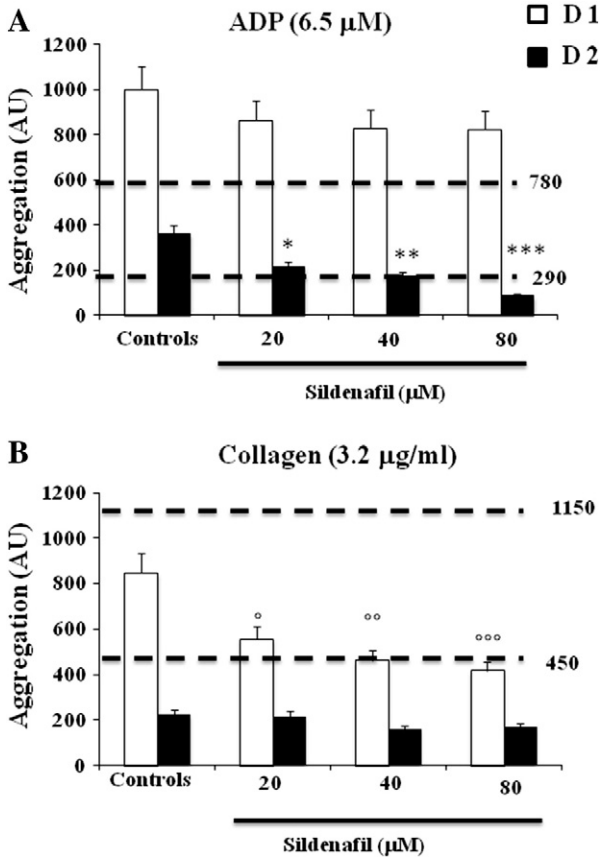

Fig. 1. Platelet aggregation determined in the whole blood by using a Multiplate aggregometer in response to 6.5 μM ADP (A) and 3.2 μg/ml collagen (B). Tests were performed in duplicate determinations. D1 indicates donors with high platelet reactivity; D2 indicates donors with low platelet reactivity. The values are expressed in arbitrary aggregation units (AU). For ADP: *p = 0.009; **p = 0.001; ***p = 0.0007. For collagen: °p = 0.01; °°p = 0.003; °°°p = 0.001.

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conditions it triggers platelet aggregation via P2Y12 receptor at mean values of 290–780 AU (arbitrary aggregation units). High platelet reactivity to ADP (values higher than 780 AU) may be a risk factor for thrombotic events, while very low platelet reactivity to ADP (values less than 290 AU) may be a risk factor for hemorrhagic disorders [10].

Interestingly, in donors whose platelets after ADP administration aggregate to a value near 290 AU, we found that sildenafil reduced aggregation at values below the physiological range (Fig. 1A). The most significant effect ($p = 0.0007$) was detectable at a concentration of 80 μM. In donors whose platelets aggregate to values higher than 780 AU, sildenafil reduced platelet aggregation maintaining these values higher than the physiological range.

Collagen is another natural platelet agonist that, under physiological conditions, triggers platelet aggregation via glycoprotein (GP) VI transmembrane receptor at mean values of 450–1150 AU. Interestingly, in donors whose platelets aggregate at physiological values we found that, at all concentrations used, sildenafil significantly reduced the aggregation induced by collagen at values below the physiological range (Fig. 1B).

This study "mimics" ex vivo what could occur in the peripheral blood after intake of sildenafil. These data, although obtained in a small cohort of young healthy subjects, seem in fact to indicate that sildenafil can reduce platelet aggregation in a dose-dependent manner. On this basis we could hypothesize that some beneficial effect of sildenafil, e.g. on pulmonary hypertension, could stem on its antithrombotic activity. Conversely, the abuse of this substance (e.g. for recreational use by young healthy men and elite athletes) could represent a risk factor for hemorrhagic disorders.

Conflict of interest statement

The authors report no relationships that could be construed as a conflict of interest.

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