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ORIGINAL ARTICLE

Circulating endothelial progenitor cells and endothelial function after chronic Tadalafil treatment in subjects with erectile dysfunction

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We evaluated the effect of a chronic treatment with Tadalafil on progenitor cells (PCs) number and endothelial function in patients with erectile dysfunction (ED) with or without cardiovascular risk factors. Twenty-six subjects with ED and 23 aged matched controls were studied. All subjects underwent blood tests, International Index of Erectile Function (IIEF-5), Nocturnal Penile Tumescence Rigidity Monitoring test (NPTRM), brachial artery flow-mediated dilation (FMD) and PCs count. International index of erectile function, FMD and PC count were re-evaluated in all subjects at the end of Tadalafil and placebo treatment. With respect to controls patients had lower basal FMD ($P<0.05$) and basal PCs ($P<0.05$). Treatment with Tadalafil determined a significant increase in PCs ($P<0.001$) and FMD ($P<0.001$) with respect to basal level. Positive correlation was found between basal FMD and PCs ($P<0.05$) and between basal FMD and PCs increase after Tadalafil treatment ($P<0.05$). Tadalafil promotes a mobilization of PCs and improves endothelial function in ED patients.
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Introduction

It is now well established that vascular endothelium has a fundamental role in the control of vascular tone and blood flow.[1] Endothelial monolayer is not just a simple barrier, but a true organ which synthesizes and releases substances playing in a paracrine and endocrine manner on vascular tone and platelet aggregation. The main vasorelaxing substance produced by endothelial cells through the activation of nitric oxide synthase (eNOS) is nitric oxide (NO). Conditions associated with a reduced endothelial function may determine an imbalance between vasodilating and vasoconstricting substances produced by or acting on vascular wall.[2]

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Epidemiological studies show that erectile dysfunction (ED) and cardiovascular diseases (CVD) share common risk factors, such as hypertension, diabetes, smoking, and hyperlipemia. Common denominator of these clinical situations is an endothelial dysfunction, characterized by a disturbance in the endothelial monolayer that triggers the atherosclerotic process.[3–6] Recently, we demonstrated that ED could exist in patients without visible vascular structural damage hypothesizing that ED may represent a symptom of an initial endothelial dysfunction.[7] In these patients, a chronic and non on-demand treatment with Tadalafil, a long half life phosphodiesterase 5 (PDE5) inhibitor, induced spontaneous resumption of erections only when minimal alteration at carotid level were present.[7] This finding suggested a role of Tadalafil on endothelial monolayer function probably due to an improvement of endothelial function.[7]

In the last years, it has been demonstrated that injured endothelial monolayer is restored by circulating bone marrow-derived endothelial progenitor cells (EPCs). These cells migrate into peripheral circulation and differentiate into mature endothelial

cells and are able to provide a circulating pool of cells that may contribute not only to endothelial repair, but also to neovascularization.[8–10]

The mobilization of stem cells from the bone marrow is a complex mechanism not yet clarified, but a key step seems to be the local secretion of metalloproteinase-9 (MMP-9) by the haematopoietic and stromal compartments of the bone marrow, and thus resulting in releasing of soluble kit ligand. This process allows progenitor cells (PCs) activation. A recent study on mice suggested the essential role of endothelial nitric oxide synthase (e-NOS), which produces NO from L-arginine, for the functional activity of haematopoietic stem cells and PCs.[11] This study also demonstrated that NO acts primarily in a paracrine manner to induce an increase in the number of circulating EPCs, and that the lack of e-NOS induces defective haematopoietic recovery and PC mobilization.[11]

Recently, we evaluated the role of Vardenafil, a selective PDE5 inhibitor, on the number of circulating EPCs and we found that Vardenafil, within 4 h, significantly increased their number.[12] Hypothesizing both a drug-induced and a class-induced effect, in the present study, we evaluated the effect of a chronic treatment with Tadalafil on PC number and endothelial function in patients affected by ED with or without cardiovascular risk factors.

Patients and methods

After approval by local ethics committee of the University of Padova, 26 consecutive patients with ED and 23 controls gave informed consent and were enrolled in this study. The patients were aged 50.1 ± 8.2 (range 35–58) and had a history of 6–12 months of ED, defined as the consistent inability to obtain and/or maintain an erection for satisfactory sexual intercourse. Erectile dysfunction was assessed by an International Index of Erectile Function (IIEF-5) value <21. The control group consisted of 23 age-matched patients (47.6 ± 5.7 years, range 39–54) with normal erectile function, without any therapy and, based on clinical history and biochemical blood exams, without cardiovascular risk factors (smoking, hypertension, diabetes, hyperlipemia, hyperhomocysteinemia, body mass index >25). Erectile dysfunction was confirmed in all patients with Nocturnal Penile Tumescence and Rigidity Monitoring (NPTRM) carried out with the RigiScan Plus Rigidity Assessment System (Dacomed, USA) performed in two consecutive nights.[13]

Vascular endothelial function was evaluated with brachial artery flow-mediated dilation (FMD) assessed in the morning, after an overnight fasting and 24 h therapeutical washout. Longitudinal B-mode ultrasound images of the brachial artery, 4–6 cm proximal to the antecubital crease were taken. Images were obtained after 10 min of supine relaxation during reactive hyperemia, which was induced by inflation for 5 min to suprasystolic pressure (≈ 20 mm Hg) of an occlusion cuff placed around the forearm. Increased blood flow and shear stress during hyperemia leads to NO-dependent FMD of the brachial artery. End-diastolic images were stored and arterial diameters were measured as the distance between the arterial wall intima–media interfaces. The FMD was defined as the maximum percent change in arterial diameter from 10 to 120 s postdeflation of the occlusion cuff.[14] Assessment of FMD was performed using a high resolution Echo-Colour-Doppler (AU5, Esaote, Genova, Italy) equipped with a 7.5 MHz linear probe with <0.2 mm axial resolution by the same doctor who was blinded to patient clinical status.

Exclusion criteria were chronic renal insufficiency, liver diseases, pelvic surgical interventions, major psychiatric diseases, Peyronies's disease. Patients with a pharmacological therapy other than the antihypertensive one were excluded from this study and no new therapy was started at the time of the visit.

Blood samples for circulating PCs count were evaluated by flow cytometry, as previously described.[15] Briefly, analysis was performed on 150 μl of peripheral blood incubated with fluorescein isothiocyanate-labeled (FITC) monoclonal antibodies against human CD34 (Becton Dickinson, Milano, Italia, EU) and allophycocyanin (APC)-labelled monoclonal antibodies against human AC133 (Miltenyi Biotec, Bergisch Gladbach, Germany, EU). Expression of the stem cell marker CD34 is also found on lower level on mature endothelial cells, so we search for AC133 which is highly expressed on immature stem cells, but whose expression is lost during the differentiation to mature endothelial cells.[16]

All ED patients were treated with Tadalafil 20 mg, one tablet three times a week for 3 months, and evaluation of brachial FMD and PC number was performed at baseline at the third day from therapy suspension. Control patients were re-evaluated for PCs number and FMD after three months of placebo treatment with the same protocol plan.

Data are expressed as mean $\pm$ s.d. Normality of the data was evaluated with the Kolmogorov–Smirnov test. Comparison of PC and FMD values between patients and controls and between baseline and the end of therapy was performed with the Wilcoxon sum of ranks test for matched or unmatched pairs, respectively. *P*-values of <0.05 were regarded as statistically significant.

Results

Table 1 shows main outcomes of patients and controls. International Index of Erectile Function evidenced normal sexual activity (score >21) in

Table 1 Major clinical data, basal FMD and PCs of patients and controls

	Patients no. 26	*Controls no. 23*
Age (years)	50.1±8.2	47.6±5.7
BMI >25	2	—
IIEF-5	17±2	24±1
BP >140/90 mm Hg	8	—
Glucose >126 mg/dl	2	—
Cholesterol >200 mg/dl	12	—
Not HDL cholesterol >120 mg/dl	14	—
Triglicerids >180 mg/dl	2	—
Homocystein >31 μmol/l	1	—
Testosterone <10 nM/l	—	—
Smokers	6	2
Basal FMD (%)	9.2±3.2	12.7±2.7
Basal PCs (cells/ml)	1309.9±440.9	1867.7±373.3

Abbreviations: BMI, body mass index; BP, blood pressure; FMD, flow-mediated dilation; IIEF-5, international index of erectile function; PCs, progenitor cells.

19 of 26 (73%) patients after Tadalafil treatment. At the end of Tadalafil and placebo treatment, we evaluated blood pressure and biochemical parameters without finding any statistical significance before and after the drugs administration (data not shown).

With respect to controls patients showed a significant reduction of basal FMD (9.2±3.2 vs 12.6±2.1%; $P<0.05$) and basal PCs (1309.9±440.9 vs 1928.6±559.5; $P<0.05$) (Figures 1 and 2).

During Tadalafil therapy no cardiovascular events occurred and three cases of side effects, which did not determine drop-out, were reported (two headache, one flushing). Side effects decreased in severity with the following drug assumption.

Oral administration of Tadalafil 20 mg three times a week for 3 months determined a significant increase in PC values with respect to baseline (2531.9±998.5 vs 1309.9±440.9, $P<0.001$) (Figure 1). Also FMD test values at the end of the therapy significantly increased with respect to baseline (17.9±5.3 vs 9.2±3.2, $P<0.001$) (Figure 2). In the control group, no significant modifications were seen between baseline and after 3 months with placebo administration both for PCs (1928.6±559.4 vs 1905.8±461.9) and FMD (12.6±2.1 vs 13.8±2.2) (Figures 1 and 2).

Positive correlation between basal FMD and PCs ($R^2=0.16$; $P<0.05$) (Figure 3) and between basal FMD and PC increase after Tadalafil ($R^2=0.16$; $P<0.05$) (Figure 4) were found. Progenitor cells number and FMD values were increased after therapy both in patients with and without overt cardiovascular risk factors. Study limitation: the study is not randomized; thus control subjects were not treated with Tadalafil and patients were not treated with placebo.

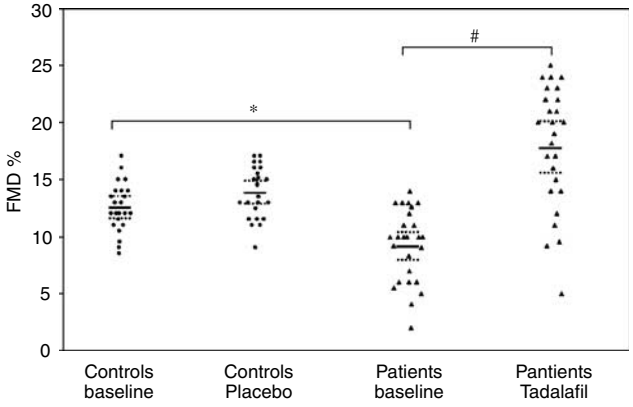

Figure 1 Flow-mediated dilation (FMD) at baseline and after therapy in patients and controls $^*P<0.05$; $^{\#}P<0.001$. *Continuous and dashed lines* indicate the mine value and the 95% confidence intervals, respectively. The mean±s.d. are as follows: controls baseline, 12.6±2.1% (range 8.5–17.0%; 95% confidence interval 11.6–13.5%); controls placebo 13.8±2.2% (range 9.0–17.0; 95% confidence interval 12.8–14.7%); patients baseline 9.2±3.2% (range 2.1–14.0%; 95% confidence interval 7.9–10.5%); patients Tadalafil 17.9±5.4% (range 5.0–25.1; 95% confidence interval 15.7–20.0%).

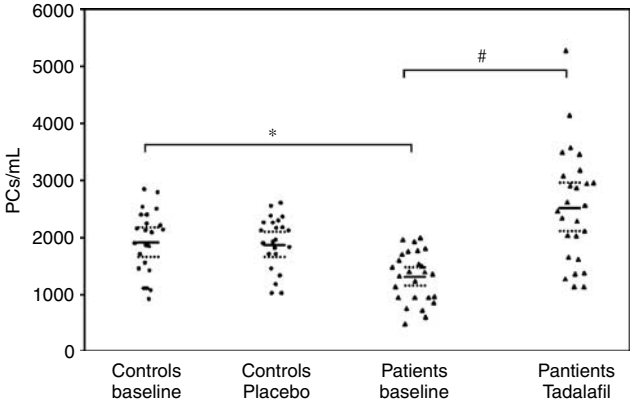

Figure 2 Progenitor cells (PCs) at baseline and after therapy in patients and controls $^*P<0.05$; $^{\#}P<0.001$. *Continuous and dashed lines* indicate the mine value and the 95% confidence intervals, respectively. The mean±s.d. are as follows: controls baseline, 1928.6±559.4 PCs/ml (range 910.0–2839.0 PCs/ml; 95% confidence interval 1686.7–2170.5 PCs/ml); controls placebo 1905.8±461.9 PCs/ml (range 1007.0–2593.0 PCs/ml; 95% confidence interval 1706.1–2105.6 PCs/ml); patients baseline 1309.9±440.9 PCs/ml (range 476.0–1995.1 PCs/ml; 95% confidence interval 1131.8–1488.0 PCs/ml); patients Tadalafil 2531.9±998.5 PCs/ml (range 1133.1–5285.0 PCs/ml; 95% confidence interval 2128.6–2935.3 PCs/ml).

Discussion

Selective PDE5 inhibitors are widely used drugs for the treatment of ED, given that PDE5 is thought to be responsible of penis detumescence. PDE5 is expressed in the corpora cavernosa of the penis, but its expression has been demonstrated in other organs such as smooth muscle, skeletal muscle,

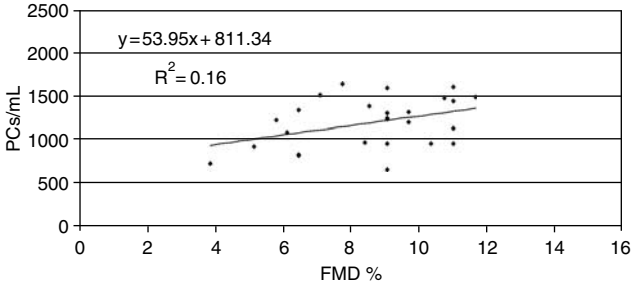

Figure 3 Positive correlation between basal flow-mediated dilation (FMD) and progenitor cells (PCs) count ($R^2 = 0.16$; $P < 0.05$).

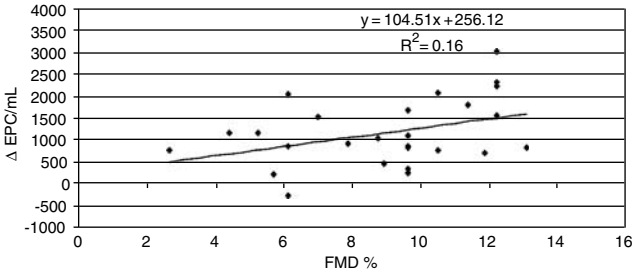

Figure 4 Positive correlation between basal flow-mediated dilation (FMD) and progenitor cells (PCs) increase after Tadalafil treatment ($R^2 = 0.16$; $P < 0.05$).

brain and kidney.[17] Nitric oxide acts by increasing the activity of guanylyl cyclase, which increases the production of cyclic guanosine monophosphate (cGMP) and PDE5 inhibitors block the breakdown of cGMP. An effect of PDE5 inhibitors not only on erectile endothelium of the corpora cavernosa, but also on other organs was suggested by a mice model evaluating a new PDE5 inhibitor, DA-8159. If chronically administered this drug is able to ameliorate an endothelial dysfunction-related vascular injury and to induce haematological and bone marrow modifications with an increase in lymphomonocytic component count and in bone marrow density.[18,19] From this animal model it is hard to estimate the effect on PCs, but they are a subset of the lymphomonocyte component of the peripheral circulation.

Recently, we demonstrated that in ED patients with or without cardiovascular risk factors the number of PCs is reduced and that their number may be increased by a PDE5 inhibitor, Vardenafil.[12,20] In this study, we observed a significant increase of endothelial function (FMD related) and circulating PCs after 3 months treatment with another PDE5 inhibitor, Tadalafil. This improvement in PCs number may result in an effective vasculoprotection preventing the initiation and progression of endothelial dysfunction. We found positive correlation between basal FMD and basal PCs number suggesting that an impaired endothelial function is correlated with a low level of circulating PCs, and thus that a normal pool of circulating PCs may be important for the health of an ED patient vascular tree. In fact, the results of the 'Endothelial Progenitor Cells in Patients with Coronary Artery Disease' (EPCAD)-Study[10] clearly demonstrated that the level of circulating $CD34^+/KDR^+$ EPCs predicts the occurrence of cardiovascular events and cardiovascular death, furthermore increased EPCs levels were associated with a lower risk for cardiovascular death, first major cardiovascular event, revascularization and hospitalization. Similar results were obtained in a patient population including healthy control subjects, patients with stable coronary artery disease, and patients with acute coronary syndrome.[21] The mechanism by which Tadalafil is able to increase PCs and to restore endothelial function is not known but at least two hypotheses may be done. The first involves the presence of PDE5 in the bone marrow that, when inhibited, may magnify the local effect of NO thus leading to the mobilization of stem and progenitor cells. Direct evidences of this mechanism are lacking. Alternatively, a peripheral action (on endothelial cells of the vascular tree) of PDE5 inhibitors could be hypothesized in the signaling pathway leading to PC activation and mobilization. In this study we also evaluated if there is a correlation between the entity of the endothelial function and the capability of Tadalafil to induce an increase in PC count. Flow-mediated dilation is the only clinical way to indirectly determine NO production by endothelium,[22] given that FMD is (1) predominantly NO-mediated (2) depressed in subjects with atherosclerosis and CV risk factors and (3) well correlated with coronary vascular endothelial function. We observed that patients with lower FMD values had a reduced increase of PCs after Tadalafil treatment. This finding suggests that patients with lower FMD have a reduced competence in mobilization and/or production of PCs, and this could probably be due to a greater alteration of the NO pathway that leads to PCs activation. Further studies are needed to clarify if in these patients a longer treatment or a dose modification of Tadalafil is able to improve the capacity to produce or mobilize PCs.

The finding that Tadalafil administration produces a mobilization of progenitor cells and improves endothelial function may have important implications for the potential role of PDE5 inhibitors in the prevention and progression of cardiovascular diseases. Therefore, ED patients with an impaired FMD test and a low PC count should be monitored because of their increased cardiovascular risk. In fact, it has been recently demonstrated that a reduced number of PC correlates with cardiovascular outcomes[10] and that endothelial dysfunction links erectile dysfunction to heart disease.[6]

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