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Circulating endothelial progenitor cells and erectile dysfunction: possibility of nutritional intervention?

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To provide an overview of molecular and cellular processes involved in erectile dysfunction (ED) with emphasis on circulating endothelial progenitor cells (EPC) and discuss possible nutraceutical means of intervention. A review of literature on Pubmed related to EPC and ED was conducted. Patients with ED appear to possess a reduced number of circulating EPC, which is associated with poor endothelial function possibly as a result of underlying low-grade inflammation. Several studies support the possibility of improving erectile function by inhibition of inflammation as well as administration of various stem cell types. One particularly interesting approach is nutraceutical supplementation to increase circulating EPC, as demonstrated in the product Stem-Kine. Interventions aimed at increasing circulating EPC may have potential in treatment of vascular ED.

KEY WORDS: Endothelial progenitor cell - Erectile dysfunction - Stem-kine.

Erectile dysfunction (ED) is characterized by the lack of ability to achieve and maintain penile erection for intercourse. It is estimated that 10-30 million Americans suffer from this condition and that 50-85% of cases are associated with conditions that affect the endothelium such as hypertension, diabetes, cardiovascular disease, and dyslipidemia.[1] Currently ED is treated by oral inhibitors of phosphodiesterase-5 (sildenafil [Viagra, Revatio], tadalafil [Cialis]and vardenafil [Levitra]), which are considered the standard of care for first-line treatment. Unfortunately, 30-40% of patients are unresponsive to therapy or do not tolerate adverse effects associated with treatment.

In addition, PDE5 inhibitors are known to possess a variety of systemic effects in numerous organ systems; therefore, the long- term effects of PDE5 inhibition are still uncertain. In fact, in 1998, the US Food and Drug Administration published a report on 130 confirmed deaths among men who received prescriptions for sildenafil citrate, in which causes of death included arrythmias, sudden cardiac death and hypotension-associated events.[2]

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Biology of erections

The penis is comprised of three erectile bodies: the corpora cavernosa, which consists of 2 parallel bodies, and underneath, wedged in between, the corpus spongiosum, which contains the urethra. These three erectile bodies are heavily vascularized and contain a large proportion of smooth muscle cells. Erection is caused by neurologically-induced relaxation of smooth muscle cells in the erectile bodies, which allows influx and accumulation of blood into the balloon-like sacs between the smooth muscle cells called sinusoids. As blood accumulates, the outflow of blood is prevented by pressure from the tunica albuginea against the venous plexus, thus causing trapping of the blood, allowing erection to occur. The process of blood accumulation due to venous trapping is termed the veno-occlusive mechanism. Thus the main contributions to maintaining an erection are arterial blood entry and its venous trapping in the cavernous bodies.

The relaxation of the smooth muscles around the arteries is caused by parasympathetic nervous activation, which induces an increase in nitric oxide (NO) production by nonadrenergic, noncholinergic nerves, as well as endothelium which lines the penile arteries and cavernosal sinusoids. Accumulation of NO increases production of cyclic guanosine monophosphate (cGMP) through activation of the enzyme guanylyl cyclase. cGMP acts as a second messenger which leads to decreased calcium uptake into the cavernous and smooth muscle cells, thus causing relaxation and hence erection.[3] Since phosphodiesterase (PDE)-5 is involved in the breakdown of cGMP, the inhibition of PDE-5 has been chosen as a pharmacological goal of medications such as Viagra (sildenafil), Cialis (tadalafil) and Levitra (vardenafil). One of the first responses to deterioration in vascular disease is erectile function, primarily due to abnormal endothelial and smooth muscle responses. Accordingly, stem cell therapy may be particularly promising in addressing this aspect, which we will discuss below.

Vascular ED

Given the complexity of the erectile process, it is not surprising that ED is multifactorial, with the main cause, some studies citing up to 85%, being vascular; however ,endocrinological and neurological aspects also play a role.[4] Specific means by which endothelial dysfunction occurs include oxidative stress in the form of the oxygen-free radical superoxide, which both enhances degradation of NO (by direct conversion to peroxynitrite), as well as by decrease of its production.[5] Uncontrolled glucose levels, as found in diabetics, are a cause of advanced glycation end products, which activate several inflammatory mechanisms that directly or indirectly are adverse to endothelial function.[6] These glycation end products can inactivate NO directly as well as induce an increased production of superoxide, which also inhibits NO. Additionally, glycation end products directly suppress synthesis of endothelial nitric oxide synthase (eNOS) by endothelial cells.

The primary effector of the molecular mechanisms described above seems to be endothelial dysfunction. Montorsi et al. put forth the "Artery Size Hypothesis," in which it was proposed that in comparison to larger vessels, smaller arteries, such as the pudendal arteries supplying blood to the penile structures, are more likely to be affected by artherosclerotic and other forms of endothelial damage. Accordingly, the argument is made that initiation of vascular disease is first identified in many cases as ED, which subsequently progresses to more advanced diseases. The authors make 4 points supporting this hypothesis, namely: a) ED and coronary artery disease should be considered as two different manifestations of the same disease process; b) Prevalence of occult coronary artery disease in ED should be low; c) Prevalence of ED in patients with coronary artery disease should be high; and d) Coronary artery disease should occur subsequently to ED in a wide variety of patients.[7]

Examining some studies that have addressed this possibility, Min et al assessed 221 patients who were referred to undergo stress myocardial perfusion single-photon, emission-computed tomography for cardiovascular concerns. In addition to the normal testing procedure, the patient erectile function was quantified by the IIEF questionnaire. Patients with ED (54.8%) had a higher level of coronary heart disease in comparison to patients without ED (43.0% vs 17.0%, respectively). Specifically, in comparison to patients without ED, ED patients had LVEF lower than 50% (24.0% vs 11.0%), shorter exercise time (8.0 vs 10.1 minutes) and lower Duke treadmill score (4.4 vs 8.4; $P<0.001$). The role of ED as an independent predictor of severe coronary heart disease was identified using multivariate analysis (odds ratio, 2.50; 95% confidence

interval, 1.24-5.04; P = 0.01).[8] In another study, 12 men with ED (IIEF-5 questionnaire score </=18) and 12 age-matched controls (IIEF-5 questionnaire score >/=21) were assessed for coronary flow velocity reserve by Doppler in the left anterior descending artery, before and during adenosine infusion. Flow velocity reserve was significantly reduced in subjects with erectile dysfunction: 2.36 versus 3.19; P=0.024. Using multivariate analysis, adjusting for age, tobacco use, systolic blood pressure, heart rate and body mass index, ED was the only significant predictor of reduced coronary flow velocity reserve, P=0.016.[9] Correlation between Framingham cardiovascular risk score and ED showed that subjects in the heart disease risk cohort with moderate/severe ED (IIEF5 5-16) had a 65% increase in relative risk for developing CHD within 10 yrs compared to those without ED (IIEF5 22-25).[10] Thus it appears that ample support exists for ED being one of the primary manifestations of atherosclerosis.

Circulating endothelial progenitor cells as rejuvenators of the vasculature

For several decades the bone marrow was studied primarily as a source of hematopoietic stem cells, giving rise to the practice of bone marrow transplantation. More recently, studies have described bone marrow as containing cells capable of inducing healing in a variety of non-hematopoietic conditions ranging from liver failure to heart failure to peripheral arterial disease. Although initially the concept was proposed that bone marrow cells have "transdifferentiation" ability, recent controversy surrounding this has led to the notion that at least some of the non-hematopoietic effects of bone marrow administration are due to its high content of endothelial progenitor cells. In 1997 Ashara et al. reported isolation of "circulating endothelial progenitor cells" (EPC) from human and animal sources.[11]

The concept that the bone marrow produces a basal number of circulating EPC which act as "repair cells" for the peripheral vasculature was suggested by several lines of reasoning. The first was that in situations of hypoxia, an increase in peripheral circulation of these cells can be observed. Ashara et al. first reported this in animal models of limb ischemia.[11] In a clinical study examining vascular trauma induced by burn (8 patients) and coronary artery bypass grafting (7 patients), a 50-fold increase in circulating EPC occurred hours after the injury, which subsided to basal levels within 48-72 hours.[12] Another study examined post-infarct increases in 26 patients, 10 stable angina controls, and 17 healthy volunteers. An increase in circulating EPC correlating with circulating VEGF was observed in post-infarct patients.[13]

Direct support for the involvement of circulating EPC in healing of endothelium comes from animal studies using bone marrow transplant chimeras to identify the origin of EPC. For example, Shi et al used canine bone marrow transplants to demonstrate that when biocompatible grafts are exposed to circulation of the recipient dog, endothelialization occurred primarily of donor-origin cells.[14] In situations of arterial damage induced by wire injury, administration of labeled EPC resulted in reduced neointimal formation in a rabbit model, with accumulation of the labeled cells at the site of injury.[15] It is believed that injured endothelium secretes chemotactic signals such as MCP-1, which are responsible, at least in part, for selective attraction of EPC to the area of injury.

Instead of administering EPC, another interesting method of evoking healing through EPC is by stimulating their release from the bone marrow. G-CSF is a cytokine conventionally used for mobilization of hematopoietic stem cells during transplantation; however, it is also a known mobilizer of EPC. In an elegant study it was demonstrated that induction of mobilization by these means causes re-endothelialization of injured arteries, as well as functional recovery.[16]

Patients with ED have lower circulating EPC

As discussed above, one of the first targets of atherosclerotic disease is the penile vasculature.[7] In general, atherosclerotic disease is correlated with decreased levels of circulating EPC. One study assessed patients without cardiovascular disease that had varying scores on the Framingham risk questionnaire. A correlation between higher risk scores and low EPC numbers was found.[17] Another study assessed the preclinical atherosclerosis marker of carotid intima-media thickness (IMT) measured by ultrasound. The investigators found a correlation between IMT and low levels of CD34+, KDR+ circulating EPC. Thus, given the hypothesis that ED is caused in many cases by atherosclerotic disease, it should not be surprising that several studies have found patients with ED as having suppressed numbers of circulating EPC.

Foresta et al. reported a lower number of cells capable of forming CFU-E in circulation of men with ED as compared with healthy controls. Their rationale was based on previous reports of cardiovascular disease affecting the penile circulation first, and given that cardiovascular disease correlates with lower EPC, they sought to verify if ED was an early manifestation of cardiovascular disease.[18] A subsequent study of 119 patients with coronary artery disease found that almost 60% had ED. Presence of ED correlated with known cardiovascular risk factors such as age, hypertension, reduced left ventricular ejection fraction (LVEF) and diabetes. Importantly, ED correlated with reduction in circulating EPC.[19] A similar correlation was found in a study comparing 30 healthy overweight men with 30 overweight men suffering from ED. Severity of ED according to the IIEF score correlated with lower numbers of EPC.[20]

Inflammation causes lower numbers of EPC in ED

Chronic inflammation has been associated with ED. For example a study of 137 men with ED found significant association between the levels of the inflammatory marker C-reactive protein (CRP) and severity of penile vascular disease as measured by penile Doppler.[21] In obese men, presence of ED was also associated with increased CRP-levels.[22] Oxidative stress is a known component in numerous inflammatory conditions. In ED, salivary 8-hydroxy-2'-deoxyguanosine, a known marker of oxidative stress, has been shown to correlate with severity of dysfunction.[23] Thus it appears that underlying chronic inflammation associated with atherosclerotic disease has a negative effect on circulating EPC, which may account for impaired endothelial repair and poor vascular function associated with ED. Supporting the inflammatory cause of EPC decrease are studies in which anti-inflammatory agents partially or fully restore EPC numbers similar to healthy controls. For example, Grisar et al. demonstrated in 28 patients with rheumatoid arthritis that a 7-day course of TNF-alpha blockade resulted in restoration of circulating EPC to values comparable to healthy age-matched controls.[24]

Given the known component of oxidative stress on inflammation, as well as the direct anti-inflammatory role of components in green tea, an investigation was conducted in 20 young smokers to determine whether green tea administration altered circulating EPC.[25] Consumption of green tea daily for 2 weeks resulted in an increase in circulating EPC, as well as improvement in endothelial function as measured by the flow mediated dilation assay (FMD). In the specific case of ED, administration of the antioxidant vitamin alpha-tocophenol resulted in IIEF improvement in a pilot group of patients resistant to PDE5 inhibitors.[26]

Thus it appears that in ED a low grade, underlying inflammation, associated with markers such as CRP, TNF-alpha, as well as oxidative stress, are associated with reduction in EPC numbers and possibly causative of pathology. In order to investigate the link between EPC and actual reversion of ED, we will discuss some studies in which administration of exogenous EPC or heterogeneous cell populations containing EPC were performed.

Reversing endothelial dysfunction with EPC

The bone marrow is recognized as a major source of EPC. Suggestive of the angiogenic potential of bone marrow was an early clinical study by Tateishi-Yuyama et al., in which 22 patients with bilateral critical limb ischemia were treated intramuscularly with autologous bone marrow mononuclear cells in randomly chosen legs, with control leg receiving peripheral blood-derived mononuclear cells.[27] Improvement in the treated legs was observed in terms of ankle brachial index, pain-free walking and ulcer healing. One of the primary characteristics of endothelial dysfunction is reduction in the flow-mediated dilation assay. In an attempt to induce neo-angiogenesis through stimulation of circulating EPC numbers, mobilization of bone marrow progenitors was performed by a 2-week administration of GM-CSF in 45 patients with peripheral arterial disease.[28] At 12 weeks not only was improvement in exercise capacity noted (as compared to pre-treatment levels), but also systemic augmentation of the flow-mediated dilation assay was reported. These data suggest the possibility that administration of cells containing EPC, such as bone marrow, as well as mobilization of endogenous bone marrow EPC may be useful in repairing/improving endothelial function. Unfortunately autologous bone marrow therapy and mobilization by agents such as GM-CSF is cost-prohibitive and has a possibility

of undue pain to the patient. In the case of autologous bone marrow, the need to perform iliac crest extraction to collect sufficient cell numbers is difficult to perform in patients that numerous times have a variety of comorbidity conditions. The use of cytokine-based mobilization, if used chronically, can lead to splenomegaly, bone marrow hyperplasia, and other adverse effects.

Nutritional modulation of EPC

Mobilization of EPC using nutritional-based approaches is particularly enticing. In a recent publication, the food supplement Stem-Kine, which is currently commercially available, has been reported to induce a doubling of EPC in circulation for over a two-week period.[29] This product is composed of ellagic acid, vitamin D3, beta 1,3 glucan, and a ferment of the bacterium, Lactobacillus fermentum. Extract of green tea, extract of goji berries, and extract of the root of astragalus are added prior to the fermentation process. While more studies are necessary to investigate whether EPC modulation by Stem-Kine is sufficient to induce a clinical effect on vascular ED, demonstration of feasibility for manipulating numbers of circulating EPC by administration of a nutritionally-based product will stimulate further investigations in this area. Advantages of nutritional modulation of the EPC compartment include ability to gradually increase circulating progenitor levels in a physiological manner without the adverse effects of agents such as G-CSF that would make chronic long-term use in healthy volunteers impossible.

Conclusion

Restoring endothelial function and reversing ED through cells, drugs and foods

Currently much evidence supports a vascular dysfunction as a cause of the majority of ED. While PDE5 inhibitors have been successful in reversing symptomology of ED, to date there are no interventions that address the root cause. The advent of sensitive methods of detecting endothelial dysfunction before clinical pathology, such as flow-mediated dilation assays and intimal-medial thickness index, combined with detection of circulating EPC, provides for the first time a means of assessing efficacy of an intervention before physical symptomology is observed. Non-invasive approaches to augmenting circulating EPC such as administration of nutritional supplements offer a new area of intervention for patients resistant to PDE5 inhibitors.

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