Inflammatory Mechanisms and Oxidative Stress in Peyronie's Disease: Therapeutic "Rationale" and Related Emerging Treatment Strategies

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Abstract: Peyronie's disease (PD) is a connective tissue disorder characterized by a fibrous plaque involving the tunica albuginea of the penis. The inelastic fibrous plaque leads to a penile curvature. Several Authors have suggested an immunological genesis of this disease, others have linked PD with Dupuytren's contracture. Signs of this disease are curvature, penile pain, penile deformity, difficulty with coitus, shortening, hinging, narrowing and erectile dysfunction. The natural history of PD and the clinical course can develop from spontaneous resolution of symptoms to progressive penile deformity and impotence. Surgical treatment is indicated when patients fail the conservative medical treatment and however, only in case of disease stabilization with a condition of impossibility of penetration. The medical treatment is indicated in the development stage of PD for at least one year after diagnosis and whenever in case of penile pain. Current non-surgical therapy includes vitamin-E, verapamil, para-aminobenzoate, propoleum, colchicine, carnitine, tamoxifen, interferons, collagenase, hyaluronidase, cortisone, pentoxifylline, superoxide dismutase, iontophoresis, radiation, extracorporeal shock wave therapy (ESWT) and the penile extender. The etiology of this fibrotic disease is not widely known, although in recent years pathophysiological knowledge has evolved and new studies propose the penile trauma as cause of the disease. The penile trauma results in a delamination of the tunica albuginea with a consequent small hematoma, then the process evolves as an inflammation with accumulation of inflammatory cells and production of reactive oxygen species (ROS). In the course of the inflammation, Peyronie's disease occurs due to the activation of nuclear factor kappa-B, that induces the production of inducible nitric oxide synthase (iNOS), with an increase of nitric oxide, leading to increased production of peroxynitrite anion. All these processes result in the proliferation of fibroblasts and myo-fibroblasts and excessive production of collagen between the layers of the tunica albuginea (penile plaque). Referring to the current knowledge of inflammatory and oxidative mechanisms of PD, a possible therapeutic strategy is then analyzed.

Keywords: Peyronie's disease, peyronie's disease treatment, oxidative stress, nitrosative stress, radical oxygen species, free radicals.

INTRODUCTION

Peyronie's disease (PD) is a connective tissue disorder characterized by a fibrous inelastic plaque involving the tunica albuginea layer of the penis. Every defect in the tunica albuginea of the penis can deform the appearance and the static of the penis resulting in a possible penile curvature. The albuginea of the corpora cavernosa consists of two layers of connettive tissue (outer longitudinal and inner circular layer) [1]. After traumatic events, these layers are separated by micro-bleeding and blood clots caused by the rupture of small vessels (which runs through the layers). For the first time, François Gigot de Peyronie (surgeon of the Court of King Louis XV of France) described this disease in 1743 [2]. Several Authors have suggested an immunological origin of this disease [3, 4]. Others authors have linked Peyronie's disease with Dupuytren's contracture, indicating considerable incidence of Dupuytren's disease in patients with PD (15.4-21%) [5, 6], while 10%-40% of patients with PD are affected by Dupuytren's contractures [7]. Ralph *et al.* found a significant association between Peyronie's disease and HLA-B27 [8]. A recent study performed on the excised tunica albuginea (apparently normal and distant from plaque) from patients with PD operated using Nesbit technique, have shown that in 61.3% of the cases the histological findings indicated fibrosis of tunica albuginea. Another similar study has detected the presence of alterations in the tissues of corpora cavernosa adjacent to the PD-plaque, consisting of histological changes (similar to those found in plaque). This seems to suggest that, in patients with PD, the noxa (phenotypic expression) is widespread within the entire tunica albuginea of the corpora cavernosa [9,10]. A recent research indicates that PD is more common in men with skin white [11] and several studies indicate a prevalence of 3.2 to 8.9% in adult men [12-14]. La Pera *et al.* showed a significant correlation between cigarette smoking and PD [13]. We believe that the discovery of this association is due to the well-known adverse effects of cigarette smoking on the antioxidant defense system [15-18]. Signs of Peyronie's disease are: penile curvature, pain, penile deformity, difficulty with coitus, shortening, hinging, narrowing and erectile dysfunction. The etiology of this fibrotic disease is not widely known, although in recent years pathophysiological knowledge has evolved and new studies propose the penile trauma (micro- or macro-trauma) as cause of the disease [19,20]. The inflammatory lesion may be the result of penile trauma during sexual intercourse (sometimes for particular positions) or caused by work-related injuries or accidents related to the everyday life. Zargooshi recently reported an increased incidence of PD after penile trauma resulting from a particular practice called "*taqaandan*": a forceful bending of erect penis that some men, in some regions of the Middle East, perform to stop abruptly penile

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erection [21, 22]. The "natural history" of patients with PD and the clinical course varies from spontaneous resolution of symptoms to progressive deformity and erectile dysfunction. Though some studies reported the spontaneous resolution in patients without any therapy [23, 24], other authors have achieved a worsening of the penile curvature, respectively, in 30,2% and 48% of cases at follow-up [25, 26]. This suggests that the patients must be informed that PD is progressive without treatment and spontaneous resolution is rare. Surgical treatment is indicated when the patients have failed conservative therapy and however, only in case of disease stabilization with a condition of impossibility of penetration due to the strong penile curvature. Medical treatment is indicated in the development stage of PD, for at least one year after initial diagnosis and whenever there is local pain.

Current conservative treatment includes: vitamin-E, verapamil, para-aminobenzoate (potaba), propoleum (propolis), colchicine, tamoxifen, interferons, collagenase, hyaluronidase, cortisone, pentoxifylline, superoxide dismutase, iontophoresis (as a vehicle for drugs into the penis), radiation and extracorporeal shock wave therapy (ESWT) [27-30] etc. Regarding the penile curvature secondary to PD, in recent years, it was proposed a non-invasive and non-surgical approach consisting in the use of a new medical device: the penile extender [31].

INFLAMMATION IN PEYRONIE'S DISEASE

It has not even been established the origin of the inflammatory process that leads to an excessive collagen deposition and then to the formation of plaque, although the most accepted theory seems to be the one that considers this fibrosis as the result of a penile trauma. The trauma would result in a delamination of the tunica albuginea with a consequent small hematoma and deposition of fibrin. Microvascular trauma leads to extravascular leakage of blood, with thrombus formation that leads to deposition of fibronectin and fibrin. Then the process evolves as an inflammation with accumulation of inflammatory cells and production of reactive oxygen species (ROS). From these effects it arises a chain of events leading to the proliferation of fibroblasts and myo-fibroblasts and excessive production of collagen between the layers of the tunica albuginea (plaque) (Fig. **1**). In Peyronie's disease the process of wound healing does not occur regularly, on the contrary in PD occurs a dysregulation of this process with an overproduction of collagen and the replacement of normal cellular material with connective tissue. The deposition of fibrin determines the start of the response to wound healing with the subsequent phlogistic process: attraction of inflammatory cells (macrophages, neutrophils, mast cells etc.) and production of inflammatory cytokines (transforming growth factor-beta-1 etc.) [32-34]. Transforming growth factor-beta (TGF-beta-1) is also released by platelets together with platelet-derived growth factor (PDGF) [35]. Neutrophils are present especially in the first 24 hours with the function of removing debris and bacteria. After 48 hours become prevailing macrophages whose function is to remove bacteria and debris and release of TGF-beta-1. In the area occurs a concentration of fibroblasts attracted by TGF-beta-1 and PDGF [36]. TGF-beta-1 induces the biosynthesis of collagen and leads to the formation of plaque; this is demonstrated both in animal models than in human [37,38]. TGF-beta-1 also stimulates the transformation of fibroblasts into myofibroblasts, inhibits myogenesis and induces osteogenesis [39,40]. Myofibroblasts are mesenchymal cells that share the phenotype of smooth muscle cells and fibroblasts and therefore possess the ability to contract and to synthesize collagen. Cantini *et al.* reported a profibrotic role of Myostatin (a member of the TGF-beta family) in Peyronie's disease [41]. Though PDGF is synthesized stored and released by platelets (as well as TGF-beta-1) upon activation, it is produced by other cells: monocytes and macrophages, vascular endothelial cells, and smooth muscle cells [42]. The fibroblasts are one of the main targets of PDFG [36]. The biological activities of PDGF are potent mitogenic effect and chemoattraction directed to fibroblast, stimulation and induction of the synthesis of collagens and proteoglycans. There are other mediators in PD who mediate migration and proliferation of fibroblasts: fibroblast growth factor (FGF), interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-alpha) [43,44] (Fig. **1**). Also FGF and IL-1 stimulate the collagen synthesis. Monocytes are the main source of secreted IL1 and normally, TNF-alpha is mainly secreted by macrophages. In Peyronie's disease production of FGF by the plaque derived myofibroblasts resulted in significantly higher [44]. TGF-beta-1, PDGF and plasminogen activator inhibitor-1 (PAI-1: profibrotic protein) are overexpressed in PD plaques [45]. Some authors reported that men with PD, in their tunica albuginea have more represented type III collagen than type I collagen [46,47]. The progression of the PD plaque may possibly lead to calcification or ossification in 15-25% of patients [37,48,49]. The calcification of the penile plaque may result from osteogenic differentiation of fibroblasts and/or myofibroblasts and may be triggered by TGF-beta-1, chronic inflammation, oxidative stress and other profibrotic factors [49].

During the early phase of PD there is an increase in oxidative stress which in the form of free radicals induces overexpression of fibrogenic cytokines, as well as augmented transcription and synthesis of collagen. TGF-beta-1, in addition to directly inhibiting collagenase and promoting collagen synthesis, increases ROS levels [50] (Fig. **1**).

ROS and TGF-beta-1 are the major mediators for enhancement of collagen synthesis, decrease in local activity of collagenase and overproduction of other extracellular matrix proteins [51]. ROS include: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), organic hydroperoxide (ROOH), alkoxy radicals (RO) and peroxy radicals (RO2), hypochlorus acid (HOCl), singlet oxygen ($^1O_2^\bullet$), as well nitric oxide radical ($NO^\bullet$) and peroxynitrite (ONOO-) also known as reactive nitrogen species (RNS) (Fig. **2**). Nitric oxide is a noncholinergic and nonadrenergic neurotransmitter of cavernosal smooth muscle relaxation responsible for erection of the penis. Conversely, in Peyronie's disease an excess of NO production (iNOS mediated) could lead to pathological consequences in the cavernosal penile tissue [52]. In the tissue of the corpora cavernosa and in myofibroblasts from patients with Peyronie's disease there is an overexpression of inducible nitric oxide synthase (iNOS) protein and peroxynitrite [43]. It was determined that in the human PD plaque, iNOS

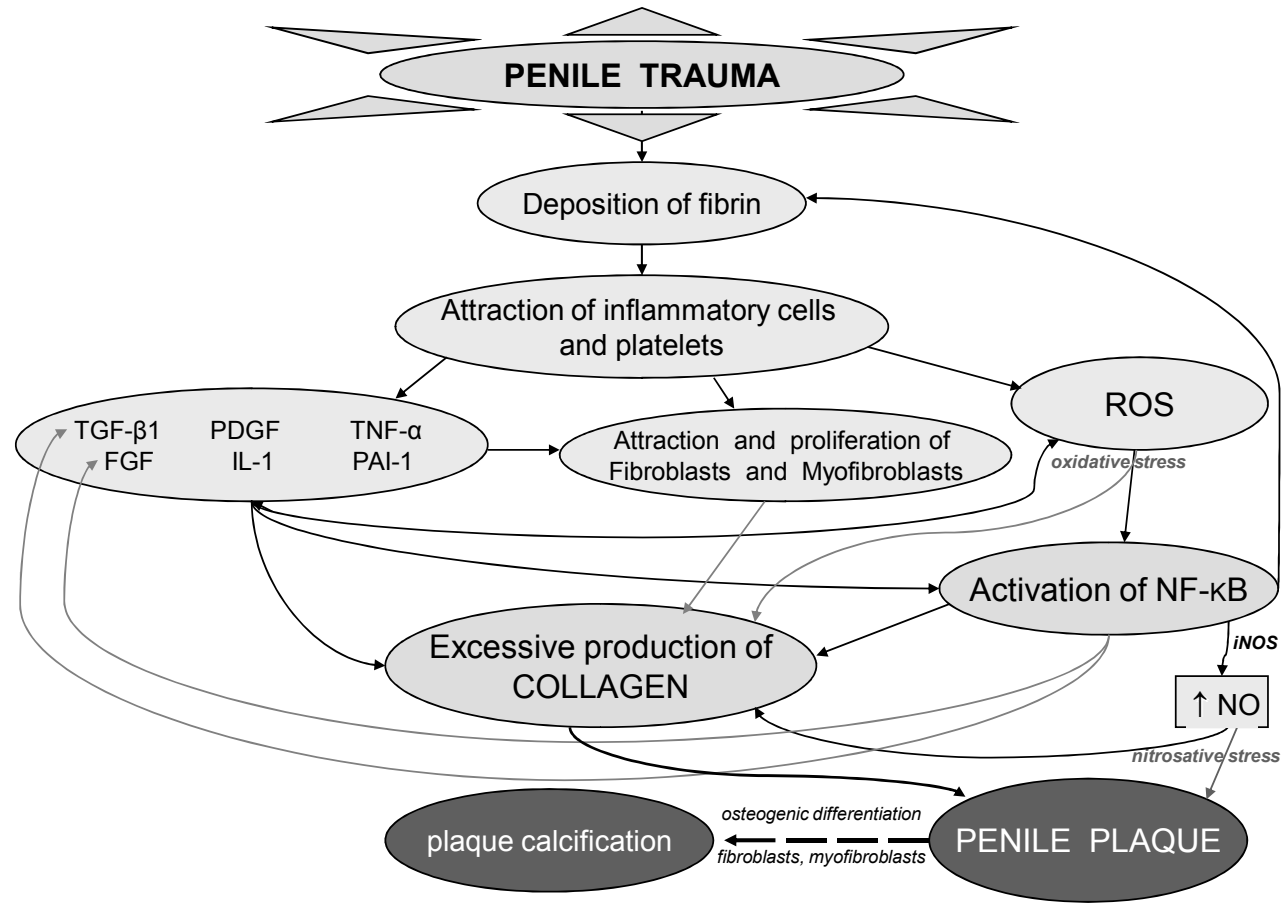

Fig. (1). Pathogenetic mechanisms of Peyronie's Disease.

mRNA protein, ROS, and the peroxynitrite marker (nitrotyrosine), were all increased in comparison to the normal tunica [51]. Since in patients with PD were found pathological levels of ROS and RNS, we can deduce that the free radical chain reaction responsible for the oxidative and nitrosative stress is the same as well as in other chronic inflammatory pathological conditions (see Fig. **2**). The oxidative stress also leads to tissue damage and increase activity in inflammatory phagocytic cells, neutrophils and macrophages. The release of cytokines and the overproduction of ROS contribute to determine the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Fig. **2**). This factor that is found in almost all cell types, is a complex protein that controls the DNA transcription; the activation of NF-κB contributes to the overproduction of collagen (penile fibrotic plaque). Sikka *et al.* described that the activation of this transcription factor leads to the expression of genes on specific targets: FGF, TGF-beta-1, iNOS, collagen, fibrin [53]. The secondary induction of the expression of iNOS leads to an increase of nitric oxide. Nitric oxide produced in response to inflammation, results in many patologic events which contribute to the appearance of fibrotic plaque [52]; the final effects of NO depend on its concentration. When concentrations of NO increase in course of inflammation, nitric oxide radical competes with superoxide dismutase (SOD) and leads to production of peroxynitrite (ONOO-) a highly toxic and pro-fibrotic compound that induces cytotoxic effects on cavernosal muscle by: lipid peroxidation, DNA fragmentation, damage and nitration of proteins resulting in cellular and organ dysfunction [43] secondary to collagen accumulation.

THERAPEUTIC LANDSCAPE AND MECHANISMS OF DRUG ACTION

Two important basic categories of pharmacological agents have been used in Peyronie's disease: anti-fibrotic drugs and anti-oxidants. Verapamil, potassium para-aminobenzoate, colchicine, interferon, tamoxifen and collagenase are used in PD as anti-fibrotic agents. However, anti-inflammatory agents and "external energy therapies" are also used.

Verapamil is a calcium channel blocker with the following activities: it reduces the local production of extracellular matrixes by fibroblasts; it reduces the proliferation of fibroblasts; it increases the local activity of collagenase; it affects the cytokine regulation of fibroblasts (reducing the excess production of fibrogenic cytokines) [54].

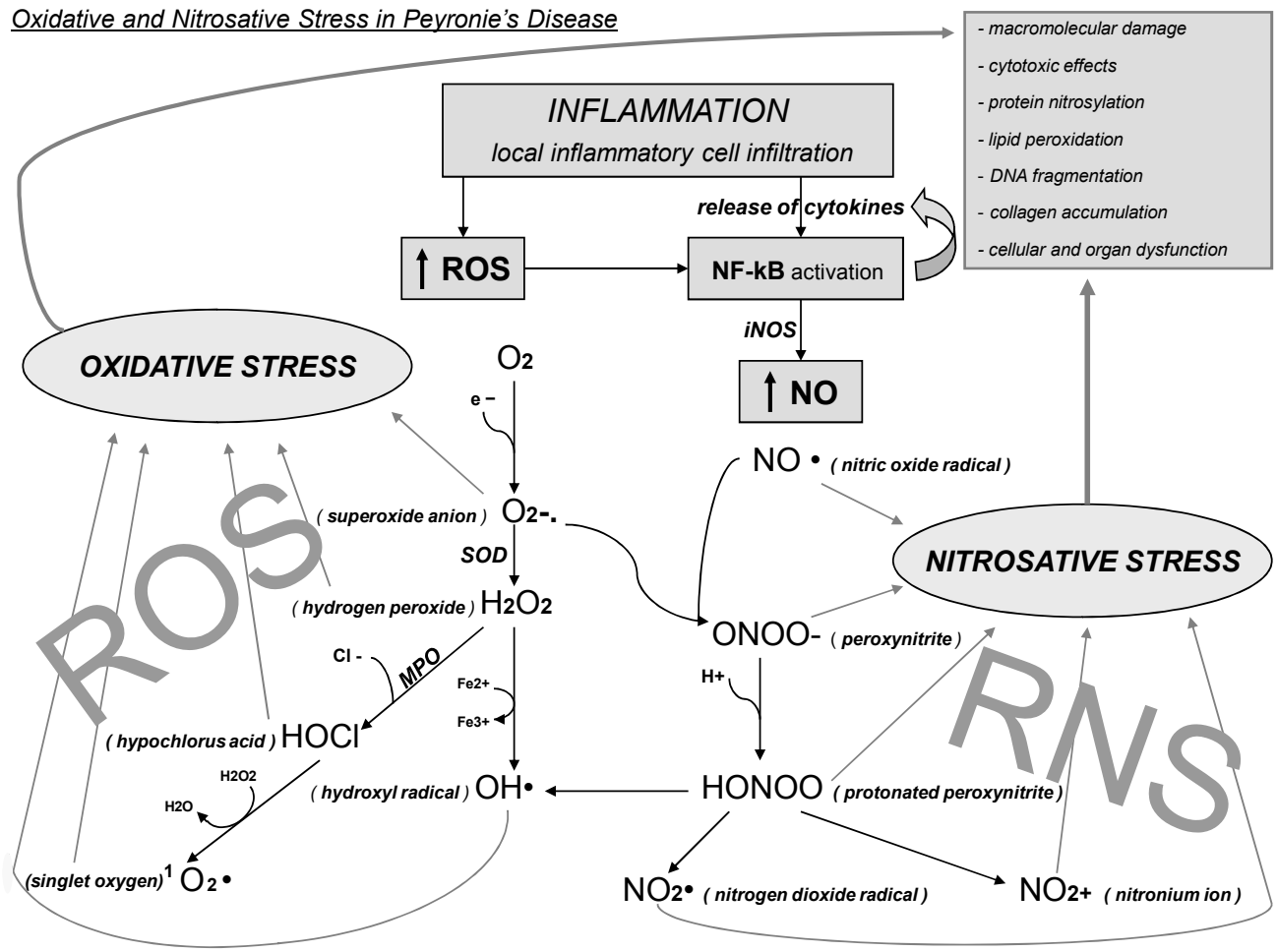

Fig. (2). Oxidative and Nitrosative Stress in Peyronie's Disease.

The results of the Levine study (intralesion injections of Verapamil/10 mg/biweekly/6-months) shows an improvement of the penile curvature in 42% of the cases and a reduction in plaque volume of ≥ 50% in 30% of the patients [55]. Rehman's research (intralesion injections of Verapamil/10-27 mg/weekly/6 months) shows a decrease of the plaque volume in 57% of the cases [56]. Even we used the Verapamil (in combination with other drugs) obtaining the following results: reduction in plaque volume = - 66.43%; improvement of penile rigidity = 63.6%; decrease of the penile curvature angle = - 14.0 degrees; pain disappearance = 100 % [57]. Other studies have not shown significant results in the use of intralesional Verapamil [58, 59]; new controlled studies are needed for a more thorough evaluation of the effectiveness of Verapamil in Peronie's disease.

Potassium para-aminobenzoate (potaba) is a member of the vitamin B-complex. In fibroblast cell cultures potaba can reduce the formation of collagen [60]. According to this *in vitro*-study it is believed that this drug decreases serotonin levels by increasing monoamine oxidase activity, resulting in enhancement of the endogenous anti-fibrotic properties of tissues. Weidner *et al.* published a prospective, placebo-controlled, randomized trial of potaba [61]. This study showed that the agent can lead to a significant reduction in plaque size, but not in curvature or pain. In conclusion the following factors represent a limitation on the use of potaba: low effectiveness against penile curvature and pain, high daily dosage required (12 grams/daily) and the possible related side effects [62-64], high cost and the unavailability in some countries. Nevertheless, some authors recommend to use potaba with the intention to prevent progression of Peyronie's disease.

Colchicine is a drug commonly used against gout attacks, however this agent inhibits fibrosis and collagen deposition primarily by inhibiting neutrophil motility and activity (by inhibiting neutrophil microtubules); it up regulates collagenase activity; it reduces inflammatory mediators. A recent randomized, placebo-controlled trial shows no difference between colchicine and placebo [65] Colchicine is associated with gastrointestinal distress, among these significant diarrhea, and less frequently aplastic anemia.

Interferon (IFN) are cytokines that modulate the normal immune system in response to the presence of foreign antigens. *In vitro* studies have shown that IFN-alpha(α) and IFN-beta(β) inhibit (dosage-dependent) fibroblast and collagen production from fibroblasts derived from Peyronie's disease plaques as well as an increase in collagenase production.

In most of studies interferon alfa-2a (IFN-α-2b) was used (intralesional injection) [66, 67]; only one study was performed (not placebo-controlled) with IFN-α-2a [68]. IFN-α-2b [at a dose of 5 x 10(6) units biweekly for 12 weeks] has been documented in a multicentre, placebo-controlled study to be significantly more effective than placebo in plaque size, decreasing penile curvature and penile pain [66]. The high cost of the drug and the possibility of significant adverse effects, including fever and other flu-like symptoms, have hindered its wide use in Peyronie's disease.

Tamoxifen is a non steroidal antagonist of the estrogen receptor that is commonly employed in patients with estrogen receptor–positive breast carcinoma. Furthermore, this drug modulates the release of TGF-beta-1 from fibroblasts and blocks TGF-beta receptors, resulting in diminished fibrogenesis and then Tamoxifen was used in the treatment of PD. The only randomized, placebo-controlled, double blind trial showed no statistical difference between tamoxifen and placebo [69].

Collagenase are enzymes which by breaking the peptide bonds are able to degrade the various compounds of collagen. Few trials were performed using this medicine [70-73]; although they have demonstrated significant improvements in disease, these are not sufficient to propose a large use of collagenase in Peyronie's disease. Further studies are necessary to confirm a beneficial response to Collagenase; in this regard, a larger scale controlled multicenter trials of Clostridial Collagenase (for injection) are currently underway in the United States and in Europe.

Hyaluronidase is an enzyme that degrades hyaluronic acid by degrading interstitial bonds. It is not possible to express an opinion on this agent because there are no published trials with the use, as a single substance, in the PD treatment.

Vitamin E was the first oral therapy proposed for the treatment of PD. It is a potent antioxidant that is thought to reduce collagen deposits within the tunica albuginea. It was found that Vitamin E and its metabolites have an anti-inflammatory and anti-cyclooxygenase-2 (COX-2) property [74,75]. The antioxidant action of Vitamin E is to interact with hydroxyl radical (donating a hydrogen atom to restore the molecule to normal inert state) [54].

A double-blinded, placebo-controlled, crossover study [76] showed no significant improvements in penile curvature or plaque size. Hashimoto *et al.* (2006) [77], in a placebo-controlled study showed no higher effect of vitamin E compared to placebo.

More recently, our research that has compared outcomes in different therapeutic groups of patients with PD (patients treated with *verapamil + flavonoids + topical diclofenac + vitamin E*; patients treated with *verapamil + flavonoids + topical diclofenac*) showed more significant improvements in the groups treated with vitamin E [57].

Propolis is a resinous substance that honey bees extract from plants (buds or tree sap). The bees use this substance to seal the small open spaces in their hives to protect from atmospheric agents, other insects and microbes. Propolis has anti-inflammatory and antioxidant properties. The principal components of this substance are flavonoids: pinocembrin, galangin, chrysin, caffeic acid phenethyl ester (CAPE), aromatic and aliphatic acids, phenols, hydrocarbons, terpenes, etc. Propolis, mainly due to its component CAPE, inhibits NF-κB activity and the production of interleukins [78-80]. CAPE is known to have antimitogenic, anticarcinogenic, ant-inflammatory, and immunomodulatory properties. In particular CAPE specifically inhibits: IL-2 gene transcription and IL-2 synthesis in stimulated T-cells [79]; TNF-alpha expression and interleukin IL-8 production [80]. The chrysin, other component of propolis, inhibits: the release of nitric oxide, TNF-alpha, IL-1β; the expressions of inducible iNOS and cyclooxygenase-2 (COX-2) [81].

Lemourt Oliva *et al.* have reported the efficacy of propoleum (300-900 mg/oral/daily/six months), in patient with PD [82-85] and they did not observe any changes in the placebo group.

Pentoxifylline (PTX) reduces ROS production and protects against tissue damage by the action of its metabolites. PTX, in addition to having antioxidant properties, exerts an anti-inflammatory and antifibrotic activity. PTX attenuates TGF-beta-1-stimulated collagen deposition [86]. PTX is also a phosphodiesterase (PDE) inhibitor that downregulates TNF-alpha release and reduces the transcriptional activity of NF-κB [87]. Preventing the activity of NF-κB, PTX contributes to the lower production of collagen.

A double-blind placebo-controlled study in men with PD, has shown that PTX (400 mg/ oral / twice daily / 6 months) was moderately effective in reducing penile curvature and plaque size [88]; however the results were statistically significant. Further placebo controlled studies are needed to definitively examine PTX for the treatment of PD.

Carnitine L-carnitine is synthesized from the essential amino acids lysine and methionine. It is produced by the body in the liver and kidneys and stored in the skeletal muscles, brain, heart, and sperm. Although mechanisms have not yet been elucidated, L-Carnitine has an antioxidant activity and it has been shown to be effective in diseases characterized by increased oxidative stress.

L-carnitine scavenges superoxide anion radical and hydrogen peroxide. Carnitine stimulates directly the gene and protein expression of haem oxygenase-1 (HO-1) endothelial constitutive nitric oxide synthase (ecNOS) that are known as antioxidant, anti-inflammatory and antiproliferative [89]. Although no double-blind placebo-controlled studies have been conducted, Biagiotti & Cavallini in their trial (2001) [90] have shown good efficacy of Acetyl-L-carnitine (1g/oral/twice daily/3 months) in reducing plaque size, penile pain and disease progression.

Q10 (Coenzyme Q10, Ubiquinon), is an endogenous antioxidant coenzyme, *in vitro*, it has been shown to decrease the expression of TGF-beta-1 [91]. A prospective, double-blind, placebo-controlled randomized clinical trial was performed by Safarinejad (2010) [92]. The results of this study, have shown that CoQ10-therapy (300mg/oral/daily/for 24 weeks) produced statistically significant improvements in plaque size, penile curvature, impaired sexual function and penile pain. Despite these excellent results, further studies are needed to confirm the beneficial effects of this substance in Peyronie's disease treatment.

Orgotein is a water-soluble metalloprotein and it is the drug version of Cu-Zn superoxide dismutase, it is obtained from bovine liver and it possesses a potent anti-inflammatory activity. Superoxide dismutase is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide scavenging free oxygen radicals. Treatment with superoxide dismutase decreases ROS generation and oxidative stress. ROS have the power to further enhance inflammation and increase fibrosis, then it has been suggested that Orgotein is active in reducing the fibrotic process associated with PD. Several studies have been performed using Orgotein (intralesional injection) [93,94] for the treatment of PD obtaining good results on pain, penile curvature and plaque size, however, no placebo-controlled study has been published. Having been occurred severe allergic reactions to the bovine superoxide dismutase, the substance has been withdrawn from commerce. It is currently marketed a superoxide dismutase of plant origin (cantaloupe melon) [95,96], it would be interesting to perform placebo-controlled trials with this new preparation. However, the substance is still not recommended for people with gluten intolerance, because the covering of the capsules consists of polymeric films of wheat gliadin matrix.

Cortison, steroid substances have been used locally by injection to prevent progression of Peyronie's disease. The use of such agents is justified by their anti-inflammatory effects *via* inhibition of phospholipase A2 (PLA2) and immunosuppression. Several Authors in their placebo controlled trials (betamethasone/triamcinolone/intralesional) [97,98] showed no statistical difference between cortico-steroids-treated and placebo groups. Corticosteroid injections are not currently recommended as an intralesional therapy for PD, due to the following reasons: local tissue atrophy and distortion of tissue planes, fibrosis, thinning of skin, immune suppression, lack of objective measures of benefit.

Iontophoresis (electromotive drug administration/ EMDA) is one of the "external energy therapies" that nevertheless requires the use of drugs. It is a non-invasive method of treatment for drug administration, that the patients can autonomously perform avoiding the use of penile injections, necessary for some types of drugs (Verapamil, Corticosteroids, Orgotein). There has been much discussion about its real effectiveness, although a study of Levine & Estrada (2003) [99] who measured Verapamil concentrations in the tunica albuginea in patients after surgical treatment of PD, showed high concentrations of Verapamil in the tunica albuginea specimens after iontophoresis administration. Three double-blind studies using iontophoresis (two placebo-controlled, and one lidocaine-controlled instead of a placebo-controlled) [100-102] were performed. There were conflicting results among them, in fact Greenfield *et al.* [100] have found not statistically significant results. However, the trials are not comparable each other, since in the three studies each researcher has used different drugs. It's obvious that if several drugs are tested, different results may occur. An important fact is demonstrated by the ability of the iontophoresis in penetrating the drug beyond the tunica albuginea. After all, iontophoresis is a safe and non-invasive treatment option that the patients can autonomously perform. Since home therapy is expected to a long time, the patients may directly buy or rent the appropriate equipment.

Local Use with Creams or Gels

Liposomally Recombinant Human Superoxide Dismutase (lrhSOD) Gel

Riedl *et al*, in a randomized placebo-controlled double-blind prospective clinical study [103], have evaluated the effectiveness of topical gel containing liposomally encapsulated recombinant human Superoxide Dismutase (lrhSOD) scavenger of free oxygen radicals. The results showed statistically significant improvements only in penile pain; statistically significant effects on decrease of plaque size or penile curvature have not been reported.

Verapamil Gel

Verapamil gel has been proposed for the local treatment of PD, however there have been no published placebo-control trials. Martin *et al.* measured Verapamil concentrations in the tunica albuginea in patients undergoing surgical treatment (penile prosthesis placement) after application of the verapamil gel to the penile shaft (for a minimum of 12 h) however, the verapamil was not detected in any of the tunica specimens examined [104].

Non-Pharmacological Methods

ESWT (*Extracorporeal Shockwave Therapy)* is another type of "external energy therapies" proposed for the treatment of PD. Its mechanism of action is still unknown but common experts' opinion is that the ESWT works by remodeling the penile plaque, resulting in a local reaction and an increased macrophage activity that leads to plaque degradation. Some authors have suggested that ESWT (as the ultrasonic treatment), by causing repeated trauma to the area of plaque, can lead to the creation of contralateral scarring of the penis resulting in "false" straightening [105, 106]. The majority of uncontrolled trials describe positive effects. Two European studies with a very detailed evaluation of symptoms after treatment with ESWT revealed no significant changes [107, 108]. Some placebo-controlled trials revealed no significant improvement in plaque size or penile curvature [109-111]. Consequently, ESWT is not currently recommended as a standard therapy for Peyronie's disease. In our opinion the only reasonable indication to ESWT is the condition of not stabilized PD with large calcified plaque (more than 30 mm), normal or slightly reduced sexual function and penile curvature compatible with coitus; ESWT treatment would serve to fragment the plaque and then to increase the surface area of contact with drugs introduced.

Radiation therapy has been used for the treatment of PD for several years. No placebo controlled studies have been published. Mullah *et al.* evaluated the effect of radiation on fibrogenic cytokine production in cells cultured from PD plaque tissue; the study has shown that radiation therapy may increase the production of fibrogenic cytokines and promote the fibrotic process [112]. Randomized multicenter clinical trials are needed to establish real effectiveness of this method. Radiation is not recommended at present for the treatment of Peyronie's disease.

Penile extender is a new medical device developed to increase penile length and recently proposed for the

treatment of penile curvature PD associated. The device is utilized for daily stretching of the penis. Scroppo *et al.* (2001) have reported the first experience in the use of penile extenders as a treatment for penile curvature associated with PD [113]. Although the study reported statistically significant results (mean reduction in penile curvature = 14 degrees) it was limited by the small sample of patients. Later several studies were published on this topic, Levine *et al.* (2008) [114] reported better results on the improvement of curvature; however, the study included a limited sample of patients. Gontero *et al.* (2009) [115] in a study of 19 patients with penile curvature with stable PD and treated with penile extender for 6 months, reported only minimal improvements in penile curvature.

EMERGING TREATMENT STRATEGIES

The antioxidants agents can hinder or block the inflammatory process at different levels. Vitamin E, in addition to having properties anti-COX-2 property, interacts with hydroxyl radical (hydroxide, the more damaging ROS). Propolis inhibits: NF-κB activity (ROS-influenced) and the production of interleukins; the release of nitric oxide, TNF-alpha, IL-1β; the expressions of inducible iNOS and COX-2. L-carnitine scavenges superoxide anion radical and hydrogen peroxide. Pentoxifylline downregulates TNF-alpha release and reduces the activity of NF-κB (ROS-influenced). CoQ10 being able to transfer electrons, it acts as an antioxidant and inhibits lipid peroxidation and protects also proteins from oxidation. Superoxide dismutase are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide scavenging free oxygen radicals.

Blueberry anthocyanins (flavonoids) inhibit NF-κB, iNOS and COX-2 expression [116, 117]. Many other plants contain a high concentration of flavonoids. Quercetin, kaempferol and isorhamnetin are contained in Ginkgo biloba [118, 119]. Quercetin suppresses COX-2 expression and PGE2 production and it has been shown to inhibit TNF-induced expression by blocking the binding of the transactivator NF-kB [120]. Quercetin and kaempferol are potent inhibitors of hydrogen peroxide (H_2O_2) production [121]. The following plants S. baicalensis, T. chinensis, S. Japonica, Mahonia fortunei, and Sophora flavescens, with their flavonoid compounds (wogonin, baicalin, trollisin I and II, isoswertisin, vitexin, quercetin, rutin, isorhamnetin, trifolirhizin etc.) have exhibited significant anti-inflammatory activity by inhibition of the production of NO and TNF-alpha [122].

Therefore, in case of PD in active phase, the more appropriate therapeutic strategy is certainly a treatment that includes antioxidants associated with other antifibrotic drugs. Following a careful review of the literature and the latest knowledge on the pathophysiology of Peyronie's disease, we suggest some key strategic points. In case of conservative treatment of PD, "monotherapy" should be avoided; instead, the "combination therapies" should be preferred in order to achieve higher success rates. The drugs to choose should be those substances that have already been tested with good results; therefore it should be choosed Verapamil [administered by injection (10 mg) twice monthly + ionthophoresis (5 mg/daily)] in combination with antioxidant agents (pentoxifylline, propolis, vitamin E, flavonoids, Q10, superoxide dismutase etc.). Verapamil should be always chosen because it is the drug most experimented for local use. Antioxidants, should be prescribed for the reason that they operate to scavenge and suppress the formation of ROS (or hindering their effects) that are the major mediators for enhancement of collagen synthesis. Our experience on the use of anti-inflammatory for local use (gel) in combination with other drugs [57] allows us to suggest the use of diclofenac. Although there is no literature on this subject except our recent study, diclofenac (creams or gel) has been shown to achieve significant results in treating inflammation of the large joints, where the joint capsule is thick on average from 1 to 4 mm. If we consider that the tunica albuginea has a thickness of 0.5 to 2 mm, we can deduce that the drug is able to penetrate into the corpora cavernosa without any particular obstacles. Since the PD is a chronic illness, short-term therapies should therefore be avoided, instead it should be choose long-term therapies (at least 6 months) in order to ensure a sufficient treatment. We think it would be interesting to implement trials concerning the benefits of the use of PDE5 inhibitors in Peyronie's disease, in fact some recent studies show that these agents may be able to reverse the fibrosis [123,124] also in humans (Tadalafil) [125]. In our opinion the indication for the application of *penile extenders* should concern patients with penile curvature (not exceeding 50 degrees) and non-active Peyronie's disease. In fact, the penile extender if used in the active phase of PD, leads to a continuous traction, undoubtedly slightly traumatic and therefore may result in an exacerbation of inflammation with secondary progression and worsening.

REFERENCES

[1] Brock, G.; Hsu, G.L.; Nunes, L.; von Heyden, B.; Lue TF. The anatomy of the tunica albuginea in the normal penis and Peyronie's disease. *J. Urol.,* **1997**, *157*(1), 276-281.

[2] De La Peyronie, F. Sur quelques obstacles qui sópposent à l'éjaculation naturelle de la semence. *Mem. Acad Royale Chir.*, **1743**, *1*, 337-342.

[3] Schiavino, D.; Sasso, F.; Nucera, E.; Alcini, E.; Gulino, G.; Milani, A.; Patriarca, G. Immunologic findings in Peyronie's disease: a controlled study. *Urology,* **1997**, *50*(5), 764-768.

[4] Nachtsheim, D.A.; Rearden, A. Peyronie's disease is associated with an HLA class II antigen, HLA-DQ5, implying an autoimmune etiology. *J. Uro.l,* **1996**, *156*(4), 1330-1334.

[5] Chilton, C.P.; Castle, W.M.; Westwood, C.A.; Pryor, J.P. Factors associated in the aetiology of peyronie's disease. *Br. J. Urol.,* **1982**, *54*(6), 748-750.

[6] Nugteren, H.M.; Nijman, J.M.; de Jong, I.J.; van Driel, M.F. The association between Peyronie's and Dupuytren's disease. *Int. J. Impot. Res.,* **2011**, *23*(4), 142-145.

[7] Vanni, A.J.; Bennett, N.E. Current treatment and management of the acute phase of Peyronies's disease. *Arch. Esp. Urol.*, **2009**, 62(8), 614-622.

[8] Ralph, D.J.; Schwartz, G.; Moore, W.; Pryor, J.P.; Ebringer, A.; Bottazzo, G.F. The genetic and bacteriological aspects of Peyronie's disease. *J. Urol.,* **1997**,*157*(1), 291-294.

[9] Nale, D.; Mićić, S.; Vuković, I.; Radosavljević, R. Induratio penis plastica--localized or diffusive fibromatosis of tunica albuginea penis? *Vojnosanit Pregl.,* **2006**, *63*(11), 939-944.

[10] Costa, WS.; Rebello, SB.; Cardoso, L.E.; Cavalcanti, A.G.; Sampaio, F.J. Stereological and biochemical analysis of muscular and connective tissue components in the penile corpus cavernosum adjacent to the fibrous plaque of Peyronie's disease. *BJU Int.,* **2009**, *103*(2), 212-216.

[11] Rhoden, E.L.; Riedner, C.E.; Fuchs, S.C.; Ribeiro, E.P.; Halmenschlager, G. A cross-sectional study for the analysis of

clinical, sexual and laboratory conditions associated to Peyronie's disease. *J. Sex. Med.,* **2010**, *7*(4 Pt 1), 1529-1537.

[12] Schwarzer, U.; Sommer, F.; Klotz, T.; Braun, M.; Reifenrath, B.; Engelmann, U. The prevalence of Peyronie's disease: results of a large survey. *BJU Int.,* **2001**, *88*(7), 727-730.

[13] La Pera, G.; Pescatori, E.S.; Calabrese, M.; Boffini, A.; Colombo, F.; Andriani, E.; Natali, A.; Vaggi, L.; Catuogno, C.; Giustini, M.; Taggi, F.; SIMONA Study Group. Peyronie's disease: prevalence and association with cigarette smoking. A multicenter population-based study in men aged 50-69 years. *Eur. Urol.,* **2001**, *40*(5), 525-530.

[14] Mulhall, J.P.; Creech, S.D.; Boorjian, S.A.; Ghaly, S.; Kim, E.D.; Moty, A.; Davis, R.; Hellstrom, W. Subjective and objective analysis of the prevalence of Peyronie's disease in a population of men presenting for prostate cancer screening. *J. Urol.,* **2004**, *171*(6 pt 1), 2350-2353.

[15] Alberg, A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology,* **2002**, *180*(2), 121-137.

[16] Dietrich, M.; Block, G.; Norkus, EP.; Hudes, M.; Traber, M.G.; Cross, C.E.; Packer, L. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol *in vivo* after adjustment for dietary antioxidant intakes. *Am. J. Clin. Nutr.,* **2003**, *77*(1), 160-166.

[17] Mezzetti. A.; Lapenna, D.; Pierdomenico, S.D.; Calafiore, A.M.; Costantini, F.; Riario-Sforza, G.; Imbastaro, T.; Neri, M.; Cuccurullo, F. Vitamins E, C and lipid peroxidation in plasma and arterial tissue of smokers and nonsmokers. Atherosclerosis, **1995**, *112*(1), 91-99.

[18] Stryker, W.S.; Kaplan, L.A.; Stein, E.A.; Stampfer, M.J.; Sober, A.; Willett, W.C. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am. J. Epidemiol.,* **1988**, *127*(2), 283-296.

[19] Devine, C.J. Jr.; Somers, K.D.; Jordan, G.H.; Schlossberg, S.M. Proposal: trauma as the cause of the Peyronie's lesion. *J. Urol.,* **1997**, *157*(1), 285-290.

[20] Jarow, J.P.; Lowe, F.C. Penile trauma: an etiologic factor in Peyronie's disease and erectile dysfunction. *J. Urol.,* **1997**, *158*(4), 1388-1390.

[21] Zargooshi, J. Trauma as the cause of Peyronie's disease: penile fracture as a model of trauma. *J. Urol.,* **2004**, 172(1), 186-188.

[22] Zargooshi, J. Sexual function and tunica albuginea wound healing following penile fracture: An 18-year follow-up study of 352 patients from Kermanshah, Iran. *J. Sex Med.,* **2009**, *6*(4), 1141-1150.

[23] Williams, J.L.; Thomas, G.G. The natural history of Peyronie's disease. *J. Urol.,* **1970**, *103*(1), 75-76.

[24] Gelbard, M.K.; Dorey, F.; James, K. The natural history of Peyronie's disease. *J. Urol.,* **1990**, *144*(6), 1376-1379.

[25] Kadioglu, A.; Tefekli, A.; Erol, B.; Oktar, T.; Tunc, M.; Tellaloglu, S. A retrospective review of 307 men with Peyronie's disease; results of a large survey. *BJU Int.,* **2001**, *88*(7), 727-730.

[26] Mulhall, J.P.; Schiff, J.; Guhring, P. An analysis of the natural history of Peyronie's disease. *J. Urol.,* **2006**, *175*(6), 2115-2118.

[27] Riedl, C.R.; Plas, E.; Engelhardt, P.; Daha, K.; Pflüger, H. Iontophoresis for treatment of Peyronie's disease. *J. Urol.,* **2000**, *163*(1), 95-99.

[28] Hauck, E.W.; Diemer, T.; Schmelz, H.U.; Weidner, W. A Critical Analysis of Nonsurgical treatment of Peyronie's disease. *Eur. Urol.,* **2006**, *49*(6), 987-997.

[29] Trost, L.W.; Gur, S.; Hellstrom, W.J. Pharmacological Management of Peyronie's Disease. *Drugs,* **2007**, *67*(4), 527-545.

[30] Taylor, F.L.; Levine, L.A. Non-surgical therapy of Peyronie's disease. *Asian J. Androl.,* **2008**, *10*(1), 79-87.

[31] Gontero, P.; Di Marco, M.; Giubilei, G.; Bartoletti, R.; Pappagallo, G.; Tizzani, A.; Mondaini N. Use of penile extender device in the treatment of penile curvature as a result of Peyronie's disease. Results of a phase II prospective study. *J. Sex Med.,* **2009**, *6*(2), 558-566.

[32] Diegelmann, R.F. Cellular and biochemical aspects of normal and abnormal wound healing: an overview. *J. Urol.,* **1997**, *157*(1), 298-302.

[33] Somers, K.D.; Dawson, D.M. Fibrin deposition in Peyronie's disease plaque. *J. Urol.,* **1997**, *157*(1), 311-315.

[34] Van de Water, L. Mechanisms by which fibrin and fibronectin appear in healing wounds: implications for Peyronie's disease. *J. Urol.,* **1997**, *157*(1), 306-310.

[35] Moreland, R.B.; Nehra, A. Pathophysiology of Peyronie's disease. *Int. J. Impot. Res.,* **2002**, *14*(5), 406-410.

[36] Gentile. V.; Modesti, A.; La Pera, G.; Vasaturo, F.; Modica, A.; Prigiotti, G.; Di Silverio, F.; Scarpa, S. Ultrastructural and immunohistochemical characterization of the tunica albuginea in Peyronie's disease and veno-occlusive function. *J. Androl.,* **1996**, *17*(2), 96-103.

[37] El-Sakka, A.I.; Hassoba, H.M.; Chui, R.M.; Bhatnagar, R.S.; Dahiya, R.; Lue, T.F. An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. *J. Urol.,* **1997**, *158*(6), 2284-2290.

[38] Zimmermann, R.P.; Feil, G.; Bock, C.; Hoeltl, L.; Stenzl, A. Significant alterations of serum cytokine levels in patients with Peyronie's disease. *Int. Braz J. Urol.,* **2008**, *34*(4), 457-466; discussion 466.

[39] Li, Y.; Foster, W.; Deasy, B.M.; Chan, Y.; Prisk, V.; Tang, Y.; Cummins, J.; Huard, J. Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. *Am. J. Pathol*., **2004**, *164*(3), 1007-1019.

[40] Vernet, D.; Nolazco, G.; Cantini, L.; Magee, T.R.; Qian, A.; Rajfer, J.; Gonzalez-Cadavid, N.F. Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for peyronie disease. *Biol. Reprod.,* **2005**, *73*(6), 1199-1210.

[41] Cantini, L.P.; Ferrini, M.G.; Vernet, D.; Magee, T.R.; Qian, A.; Gelfand, R.A.; Rajfer, J.; Gonzalez-Cadavid, N.F. Profibrotic role of myostatin in Peyronie's disease. *J. Sex Med.,* **2008**, *5*(7), 1607-1622.

[42] Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell,* **1986**, *46*(2), 155-169.

[43] Bivalacqua, T.J.; Champion, H.C.; Hellstrom, W.J. Implications of nitric oxide synthase isoforms in the pathophysiology of Peyronie's disease. *Int. J. Impot. Res.,* **2002**, *14*(5), 345-352.

[44] Mulhall, J.P.; Thom, J.; Lubrano, T.; Shankey, T.V. Basic fibroblast growth factor expression in Peyronie's disease. *J. Urol.,* **2001**, *165*(2), 419-423.

[45] Davila, H.H.; Magee, T.R.; Zuniga, F.I.; Rajfer, J.; Gonzalez-Cadavid, N.F. Peyronie's disease associated with increase in plasminogen activator inhibitor in fibrotic plaque. *Urology,* **2005**, *65*(4), 645-648.

[46] Luangkhot, R.; Rutchik, S.; Agarwal, V.; Puglia, K.; Bhargava, G.; Melman, A. Collagen alterations in the corpus cavernosum of men with sexual dysfunction. *J. Urol*., **1992**, *148*(2 Pt 1), 467-471.

[47] Somers, K.D.; Sismour, E.N.; Wright, G.L. Jr.; Devine, C.J. Jr.; Gilbert, D.A.; Horton, C.E. Isolation and characterization of collagen in Peyronie's disease. *J. Urol.,* **1989**, *141*(3), 629-631.

[48] Gonzalez-Cadavid, N.F.; Rajfer, J. Molecular and cellular aspects of the pathophysiology of Peyronie's disease. *Drug Discov. Today Dis. Mech.,* **2004**, *1*, 99-104.

[49] Vernet, D.; Nolazco, G.; Cantini, L.; Magee, T.R.; Qian, A.; Rajfer, J.; Gonzalez-Cadavid N.F. Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for peyronie disease. *Biol. Reprod.,* **2005**, *73*(6), 1199-1210.

[50] Gonzalez-Cadavid, N.F.; Magee, T.R.; Ferrini, M.; Qian, A.; Vernet, D.; Rajfer, J. Gene expression in Peyronie's disease. *Int. J. Impot. Res.,* **2002**, *14*(5), 361-374.

[51] Ferrini, M.G.; Vernet, D.; Magee, T.R.; Shahed, A.; Qian, A.; Rajfer, J.; Gonzalez-Cadavid, N.F. Antifibrotic role of inducible nitric oxide synthase. *Nitric Oxide,* **2002**, *6*(3), 283-294.

[52] Rajasekaran, M.; Hellstrom, W.J.; Sikka, S.C. Nitric oxide induces oxidative stress and mediates cytotoxicity to human cavernosal cells in culture. *J. Androl.,* **2001**, *22*(1), 34-39.

[53] Sikka, S.C.; Hellstrom, W.J.G. Role of oxidative stress and antioxidants in Peyronie's disease. *Int. J. Impot. Res.,* **2002**, *14*(5), 353-360.

[54] Taylor, F.L.; Levine, L.A. Non-surgical therapy of Peyronie's disease. *Asian J. Androl.,* **2008**, *10*(1), 79-87.

[55] Levine, L.A.; Merrick, P.F.; Lee, R.C. Intralesional verapamil injection for the treatment of Peyronie's disease. *J. Urol.,* **1994**, *151*(6), 1522-1524.

[56] Rehman, J.; Benet, A.; Melman, A. Use of intralesional verapamil to dissolve Peyronie's disease plaque: a long-term single-blind study. *Urology,* **1998**, *51*(4), 620-626.

[57] Paulis, G.; D'Ascenzo, R.; Nupieri, P.; De Giorgio, G.; Orsolini, G.; Brancato, T.; Alvaro, R. Effectiveness of antioxidants (propolis, blueberry, vitamin E) associated with verapamil in the medical management of Peyronie's disease: a study of 151 cases. *Int. J. Androl.,* **2011**, doi: 10.1111/j.1365-2605.2011.01219.x. [Epub ahead of print].

[58] Arena, F.; Peracchia, G.; Di Stefano, C.; Passari, A.; Larosa, M.; Cortellini, P. Clinical effects of verapamil in the treatment of Peyronie's disease. *Acta Biomed. Ateneo. Parmense,* **1995**, *66*(6), 269-272.

[59] Nicolai, M.; Cipollone, G.; Iantorno, R.; Mastroprimiano, A.Z.; Tenaglia, R. Intralesional verapamil injection versus placebo in Peyronie's disease. *J. Urol.,* **1998**, *159,* 117 (abstract no. 450).

[60] Zarafonetis, C.J.; Horrax, T.M. Treatment of Peyronie's disease with potassium para-aminobenzoate (potaba). *J. Urol.,* **1959**, *81*(6), 770-772.

[61] Weidner, W.; Hauck, E.W.; Schnitker, J.; Peyronie's Disease Study Group of Andrological Group of German Urologists. Peyronie's Disease Study Group of Andrological Group of German Urologists. Potassium paraaminobenzoate (POTABA) in the treatment of Peyronie's disease: a prospective, placebo-controlled, randomized study. *Eur. Urol.,* **2005**, *47*(4), 530-5; discussion 535-6.

[62] Taylor, F.L.; Levine, L.A. Non-surgical therapy of Peyronie's disease. *Asian J Androl.,* **2008**, *10*(1), 79-87.

[63] Roy, J.; Carrier, S. Acute hepatitis associated with treatment of Peyronie's disease with potassium para-aminobenzoate (Potaba). *J. Sex Med.,* **2008**, *5*(12), 2967-2969.

[64] Mesnil, A.; Lewden, B.; Dumortier, J.; Cuche, M.; Euvrard, P.; Dorez, D.; Vial, T. Liver injury due to potassium para-aminobenzoate (Potaba). *Gastroenterol. Clin. Biol.,* **2004**, *28*(12), 1295-1296.

[65] Safarinejad, M.R. Therapeutic effects of colchicine in the management of Peyronie's disease: a randomized double-blind, placebo-controlled study. *Int J Impot Res.,* **2004**, *16*(3), 238-243.

[66] Hellstrom, W.J.; Kendirci, M.; Matern, R.; Cockerham, Y.; Myers, L.; Sikka, S.C.; Venable, D.; Honig, S.; McCullough, A.; Hakim, L.S.; Nehra, A.; Templeton, L.E.; Pryor, J.L. Single-blind, multicenter, placebo controlled, parallel study to assess the safety and efficacy of intralesional interferon alpha-2B for minimally invasive treatment for Peyronie's disease. *J. Urol.,* **2006**, *176*(1), 394-398.

[67] Judge, J.S.; Wisniewski, Z.S. Intralesional interferon in the treatment of Peyronie's disease: a pilot study. *Br. J. Urol.,* **1997**, 79: 40-42.

[68] Polat, O.; Gül, O.; Ozbey, I.; Ozdikici, M.; Bayraktar, Y. Peyronie's disease: intralesional treatment with interferon alpha-2A and evaluation of the results by magnetic resonance imaging. *Int. Urol. Nephrol.,* **1997**, *29*(4), 465-471.

[69] Teloken, C.; Rhoden, E.L.; Grazziotin, T.M.; Ros, C.T.; Sogari, P.R.; Souto, C.A. Tamoxifen versus placebo in the treatment of Peyronie's disease. *J. Urol.,* **1999**, *162*(6), 2003-2005.

[70] Gelbard, M.K.; James, K.; Riach, P.; Dorey, F. Collagenase versus placebo in the treatment of Peyronie's disease: a double-blind study. *J. Urol.,* **1993**, *149*(1), 56-58.

[71] Glina, S.; Gelbard, M.K.; Akkus, E.; Jordan, G.H.; Levine, L.A. The use of collagenase in the treatment of Peyronie's disease M.K. Gelbard, A. Lindner, and J.J. Kaufman. *J. Sex Med.,* **2007**, *4*(5), 1209-1213.

[72] Jordan, G.H. The use of intralesional clostridial collagenase injection therapy for Peyronie's disease: a prospective, single-center, non-placebo-controlled study. *J. Sex Med.,* **2008**, *5*(1), 180-187.

[73] Del Carlo, M.; Cole, A.A.; Hart, S.G.E.; Levine, L.A. Comparative analysis of collagen degradation in Peyronie's disease plaque and Dupuytren's contracture cord tissues injected with mixed collagenase subtypes. *J. Urol.*, **2009**, *181*(4 Suppl 1):S279-279.

[74] Jang. Q.; Yin, X.; Lill, M.A.; Danielson, M.L.; Freiser, H.; Huang, J. Long-chain carboxychromanols, metabolites of vitamin E, are potent inhibitors of cyclooxygenases. *Proc. Natl. Acad Sci.,* USA, **2008**, *105*(51), 20464-20469.

[75] O'Leary, K.A.; de Pascual-Tereasa, S.; Needs, P.W.; Bao, Y.P.; O'Brien, NM.; Williamson, G. Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. *Mutat. Res.,* **2004**, *551*(1-2), 245-254.

[76] Pryor, J.P.; Farrell, C.F. Controlled clinical trial of vitamin E in Peyronie's disease. *Prog. Reprod. Biol.,* **1983**, *9,* 41-45.

[77] Hashimoto, K.; Hisasue, S.; Kato, R.; Kobayashi, K.; Shimizu, T.; Tsukamoto, T. Outcome analysis for conservative management of Peyronie's disease. *Int. J. Urol.,* **2006**, *13*(3), 244-247.

[78] Natarajan, K.; Singh, S.; Burke, T.R. Jr.; Grunbergerm, D.; Aggarwal, B.B. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc. Natl. Acad Sci. USA,* **1996**, *93*(17), 9090-9095.

[79] Marquez, N.; Sancho, R.; Macho, A.; Calzado, M.A.; Fiebich, B.L.; Munoz, E. Caffeic acid phenethyl ester inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF-kappaB transcription factors. *J. Pharmacol. Exp. Ther.,* **2004**, *308*(3), 993-1001.

[80] Song, J.J.; Cho, J.G.; Hwang, S.J.; Cho, C.G.; Park, S.W.; Chae, S.W. Inhibitory effect of caffeic acid phenethyl ester (CAPE) on LPS-induced inflammation of human middle ear epithelial cells. *Acta Otolaryngol.,* **2008**, *128*(12), 1303-1307.

[81] Ha, S.K.; Moon, E.; Kim, S.Y. Chrysin suppresses LPS-stimulated proinflammatory responses by blocking NF-κB and JNK activations in microglia cells. *Neurosci. Lett.,* **2010**, *485*(3), 143-147.

[82] Lemourt Oliva, M.; Rodriguez Barroso, A.; Puente Guillen, M.; Vega Guerrero, C.; Navarro Cutino, M.; Perez Monzon, A. Propoleum and Peyronie's disease. *Arch. Esp. Urol.,* **2003**, *56*(7), 805-813.

[83] Lemourt Oliva, M.; Fragas Valdes, R.; Bordonado Ramirez, R.; Santana, J.L.; Gonzalez Oramas, E.; Merino, A. Peyronie's disease. Evaluation of 3 therapeutic modalities: propoleum, laser and simultaneous propoleum-laser. *Arch Esp. Urol.,* **2005**, *58*(9), 931-935.

[84] Lemourt Oliva, M.; Filgueiras Lopez, E.; Rodrıguez Barroso, A.; Gonzalez Oramas, E.; Bordonado, R. Clinical evaluation of the use of propoleum in Peyronie's disease. *Arch Esp. Urol.,* **1998**, *51*(2), 171-176.

[85] Lemourt Oliva, M.; Rodriguez Barroso, A.; Bordonado Ramirez, R.; Gonzalez Oramas, E.; Molina Castillo, F. Study of propoleum dosage in Peyronie's disease. *Arch Esp. Urol.,* **2003**, *56*(7), 814-819.

[86] Shindel, AW.; Lin, G.; Ning, H.; Banie, L.; Huang, Y.C.; Liu, G.; Lin, C.S.; Lue, T.F. Pentoxifylline attenuates transforming growth factor-β1-stimulated collagen deposition and elastogenesis in human tunica albuginea-derived fibroblasts part 1: impact on extracellular matrix. *J. Sex Med.,* **2010**, *7*(6), 2077-2085.

[87] Deree, J.; Martins, J.O.; Melbostad, H.; Loomis, W.H.; Coimbra, R. Insights into the regulation of TNF-alpha production in human mononuclear cells: the effects of non-specific phosphodiesterase inhibition. *Clinics (Sao Paulo).,* **2008**, *63*(3), 321-328.

[88] Safarinejad, M.R.; Asgari, M.A.; Hosseini, S.Y.; Dadkhah, F. A double-blind placebo-controlled study of the efficacy and safety of pentoxifylline in early chronic Peyronie's disease. *BJU Int.,* **2010**, *106*(2), 240-248.

[89] Calò, L.A.; Pagnin, E.; Davis, P.A.; Semplicini, A.; Nicolai, R.; Calvani, M.; Pessina, A.C. Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. *Int. J. Cardiol.,* **2006**, *107*(1), 54-60.

[90] Biagiotti, G.; Cavallini, G. Acetyl-L-carnitine *vs* tamoxifen in the oral therapy of Peyronie's disease: a preliminary report. *BJU Int.,* **2001**, *88*(1), 63-67.

[91] Choi, H.K.; Pokharel, Y.R.; Lim, S.C.; Han, H.K.; Ryu, C.S.; Kim, S.K.; Kwak, M.K.; Kang, K.W. Inhibition of liver fibrosis by solubilized coenzyme Q10: Role of Nrf2 activation in inhibiting transforming growth factor-beta1 expression. *Toxicol. Appl. Pharmacol.,* **2009**, *240*(3), 377-384.

[92] Safarinejad, M.R. Safety and efficacy of coenzyme Q10 supplementation in early chronic Peyronie's disease: a double-blind, placebo-controlled randomized study. *Int. J. Impot. Res.,* **2010**, *22*(5), 298-309.

[93] Gustafson, H.; Johansson, B.; Edsmyr, F. Peyronie's disease: experience of local treatment with Orgotein. *Eur. Urol.,* **1981**, *7*(6), 346-348.

[94] Primus, G. Orgotein in the treatment of plastic induration of the penis (Peyronie's disease). *Int. Urol. Nephrol.*, **1993**, *25*(2), 169-172.

[95] Vouldoukis, I.; Lacan, D.; Kamate, C.; Coste, P.; Calenda, A.; Mazier, D.; Conti, M.; Dugas, B. Antioxidant and anti-inflammatory properties of a Cucumis melo LC. extract rich in superoxide dismutase activity. *J. Ethnopharmacol.,* **2004**, *94*(1), 67-75.

[96] Skarpanska-Stejnborn, A.; Pilaczynska-Szczesniak, L.; Basta, P.; Deskur-Smielecka, E.; Woitas-Slubowska, D.; Adach, Z. Effects of oral supplementation with plant superoxide dismutase extract on selected redox parameters and an inflammatory marker in a 2,000-m rowing-ergometer test. *Int. J. Sport. Nutr. Exerc. Metab.,* **2011**, *21*(2), 124-134.

[97] Cipollone, G.; Nicolai, M.; Mastroprimiano, G.; Iantorno, R.; Longeri, D.; Tenaglia R. Betamethasone versus placebo in Peyronie's disease. *Arch Ital. Urol. Androl.,* **1998**, *70*(4), 165-168.

[98] Williams, G.; Green, N.A. The non-surgical treatment of Peyronie's disease. *Br. J. Urol.,* **1980**, *52*(5), 392-395.

[99] Levine, L.A.; Estrada, C.R.; Shou, W.; Cole, A. Tunica albuginea tissue analysis after electromotive drug administration. *J. Urol.,* **2003**, *169*(5), 1775-1778.

[100] Greenfield, J.M.; Shah, S.J.; Levine, L.A. Verapamil versus saline in electromotive drug administration for Peyronie's disease: a double-blind, placebo controlled trial. *J. Urol.,* **2007**, *177*(3), 972-975.

[101] Montorsi, F.; Salonia, A.; Guazzoni, G.; Barbieri, L.; Colombo, R.; Brausi, M.; Scattoni, V.; Rigatti, P.; Pizzini, G. Transdermal electromotive multi-drug administration for Peyronie's disease: preliminary results. *J. Androl.,* **2000**, *21*(1), 85-90.

[102] Di Stasi, S.M.; Giannantoni, A.; Stephen, R.L.; Capelli, G.; Giurioli, A.; Jannini, E.A.; Vespasiani, G. A prospective, randomized study using transdermal electromotive administration of verapamil and dexamethasone for Peyronie's disease. *J. Urol.,* **2004**, *171*(4), 1605-1608.

[103] Riedl, C.R.; Sternig, P.; Gallé, G.; Langmann, F.; Vcelar, B.; Vorauer, K.; Wagner, A.; Katinger, H.; Pflüger, H. Liposomal recombinant human superoxide dismutase for the treatment of Peyronie's disease: a randomized placebo-controlled double-blind prospective clinical study. *Eur. Urol.,* **2005**, *48*(4), 656-661.

[104] Martin, D.J.; Badwan, K.; Parker, M.; Mulhall, J.P. Transdermal application of verapamil gel to the penile shaft fails to infiltrate the tunica albuginea. *J. Urol.,* **2002**, *168*(6), 2483-2485.

[105] Levine, L.A. Review of current nonsurgical management of Peyronie's disease. *Int. J. Impot. Res.,* **2003**, *15* (Suppl 5), S113-20.

[106] Frank, I.N.; Scott, W.W. The ultrasonic treatment of Peyronie's disease. *J. Urol.,* **1971**, *106*(6), 83-87.

[107] W.Hauck, E.W.; Hauptmann, A.; Bschleipfer, T.; Schmelz, H.U.; Altinkilic, B.M.; Weidner, W. Questionable efficacy of extracorporeal shock wave therapy in Peyronie's disease: results of a prospective approach. *J. Urol.,* **2004**, *171*(1), 296-299.

[108] Strebel, R.T.; Suter, S.; Sautter, T.; Hauri, D. Extracorporeal shock wave therapy for Peyronie's disease does not correct penile deformity. *Int. J. Impot. Res*., **2004**, 16: 448-451.

[109] Hauck, E.W.; Hatzichristodoulou, G.; Lahme, S. *ESWT in Peyronie's disease*. In: *Therapeutic energy applications in Urology*. Chaussy C, Haupt G, Jocham D, Köhrmann KU, Wilbert D, editors. Georg Thieme Verlag: Stuttgart, Germany, **2005**, pp.137-143.

[110] Hatzichristodoulou, G.; Meisner, C.; Stenzyl, A.; Lahme, S. Efficacy of extracorporeal shock wave therapy on plaque size and sexual function in patients with Peyronie's Disease – results of a prospective, randomized, placebo-controlled study. Annual Meeting of the American Urological Association, 2007, May 19-24, 2007, Anaheim, California, USA. *J. Urol.,* **2007**, *177*(4 Suppl): Abstract 747.

[111] Palmieri, A.; Imbimbo, C.; Longo, N.; Fusco, F.; Verze, P.; Mangiapia, F.; Creta, M.; Mirone, V. A first prospective, randomized, double-blind, placebo-controlled clinical trial evaluating extracorporeal shock wave therapy for the treatment of Peyronie's disease. *Eur. Urol.,* **2009**, *56*(2), 363-369.

[112] Mulhall, J.P.; Branch, J.; Lubrano, T.; Shankey, T.V. Radiation increases fibrogenic cytokine expression by Peyronie's disease fibroblasts. *J. Urol.,* **2003**, *170*(1), 281-284.

[113] Scroppo, F.I.; Mancini, M.; Maggi, M,; Colpi, G.M: Can an external penis stretcher reduce Peyronie's penile curvature? *Int. J. Impot. Res.,* **2001**, *13*(Suppl 4) S21.

[114] Levine, L.A.; Newell, M.; Taylor, F.L. Penile traction therapy for treatment of Peyronie's disease: a single-center pilot study. *J. Sex Med.,* **2008**, *5*(6): 1468-1473.

[115] Gontero, P.; Di Marco, M.; Giubilei, G.; Bartoletti, R.; Pappagallo, G.; Tizzani, A.; Mondaini, N. Use of penile extender device in the treatment of penile curvature as a result of Peyronie's disease. Results of a phase II prospective study. *J. Sex Med.,* **2009**, *6*(2), 558-566.

[116] Karlsen, A.; Retterstøl, L.; Laake, P.; Paur, I.; Kjølsrud-Bøhn, S.; Sandvik, L.; Blomhoff, R. Anthocyanins inhibit nuclear factor-kappa B activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *J. Nutr.,* **2007**, *137*(8), 1951-1954.

[117] Wang, Q.; Xia, M.; Liu, C.; Guo, H.; Ye, Q.; Hu, Y.; Zhang, Y.; Hou, M.; Zhu, H.; Ma, J.; Ling, W. Cyanidin-3-O-beta-glucoside inhibits iNOS and COX-2 expression by inducing liver X receptor alpha activation in THP-1 macrophages. *Life Sci.,* **2008**, *83*(5-6), 176-184.

[118] Wang, Y.; Cao, J.; Zeng, S. Involvement of P-glycoprotein in regulating cellular levels of Ginkgo flavonols: quercetin, kaempferol, and isorhamnetin. *J. Pharm. Pharmacol.*, **2005**, 57(6): 751-758.

[119] Zhao, Y.; Wang, L.; Bao, Y.; Li, C. A sensitive method for the detection and quantification of ginkgo flavonols from plasma. *Rapid Commun. Mass Spectrom.,* **2007**, *21*(6), 971-981.

[120] Xiao, X.; Shi, D.; Liu, L.; Wang, J.; Xie, X.; Kang, T.; Deng, W. Quercetin suppresses cyclooxygenase-2 expression and angiogenesis through inactivation of P300 signaling. *PLoS One.*, **2011**, *6*(8), e22934. [Epub ahead of print].

[121] Sun, B.; Sun, G.B.; Xiao, J.; Chen, R.C.; Wang, X.; Wu, Y.; Cao, L.; Yang, Z.H.; Sun, X.B. Isorhamnetin inhibits H(2) O(2) -induced activation of the intrinsic apoptotic pathway in H9c2 cardiomyocytes through scavenging reactive oxygen species and ERK inactivation. *J. Cell Biochem.,* **2011**, doi: 10.1002/jcb.23371. [Epub ahead of print]

[122] Zhang, L.; Ravipati, A.S.; Koyyalamudi, S.R.; Jeong, S.C.; Reddy, N.; Smith, P.T.; Bartlett, J.; Shanmugam, K.; Münch, D.G.; Wu, M.J. Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid compounds. *J. Agric. Food Chem.,* **2011**, doi**:** 10.1021/jf203146e [Epub ahead of print]

[123] Ferrini, M.G.; Kovanecz, I.; Nolazco, G.; Rajfer, J.; Gonzalez-Cadavid, N.F. Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int.,* **2006**, *97*(3), 625-633.

[124] Gonzalez-Cadavid, N.F.; Rajfer, J. Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. *Nat. Rev. Urol.,* **2010**, *7*(4), 215-221.

[125] Chung, E.; Deyoung, L.; Brock, G.B. The Role of PDE5 Inhibitors in Penile Septal Scar Remodeling: Assessment of Clinical and Radiological Outcomes. *J. Sex Med.,* **2011**, *8*(5), 1472-1477.

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