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Review

Role of Oxidative Stress in the Pathophysiological Mechanism of Erectile Dysfunction

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Erectile dysfunction (ED) is defined as the inability to achieve or maintain erections sufficient for satisfactory sexual intercourse (NIH Consensus Conference, 1993). It is estimated that the prevalence of ED will double in the next 25 years. Normal erectile function depends on a precise balance between psychological, hormonal, neurological, vascular, and cavernosal factors. Therefore, an alteration in any one or combination of these factors may lead to ED. For many years, the complex interaction between the origin of impulse and the normal erectile response was not clearly understood. It was not until the early 1990s when the roles of central and peripheral phenomena in the normal erectile response was proposed. The identification of the roles of various mediators, as well as their interactions, in normal erectile function is a major development in the study of ED.

Production of nitric oxide (NO) plays a central physiological role in erections. The endothelium is the primary source of NO (Burnett, 1997). The pathophysiological mechanism of endothelial dysfunction is multifactorial, and the major outcome is impaired release of NO, which leads to ED. Although NO-mediated relaxation plays a central role in erections, other mediators, such as prostaglandins, endothelin, and bradykinins, are also important in maintaining penile tone.

Researchers have made great strides in the past two decades in identifying the pathophysiological mechanism in ED. However, the precise pathophysiological mechanism is still unclear.

There is a growing interest among researchers regarding the role of oxidative stress in the pathophysiological mechanism of ED. Oxidative stress occurs when there is an imbalance between pro-oxidants and the ability of the antioxidants to scavenge excess reactive oxygen species. The role of oxidative stress and reactive oxygen species has been extensively evaluated in the pathophysiological mechanisms of male and female infertility. However, its role in ED has not been investigated comprehensively. Initial reports from both in vitro and in vivo studies have shown a significant association between the production of reactive oxygen species and erectile dysfunction, especially in diabetic animal models.

In this article, we discuss the role of oxidative stress in the pathophysiological mechanism of ED and the therapeutic interventions and preventive strategies that may be beneficial in restoring endothelial function and normal erectile function.

Pathophysiological Mechanism of ED

Prevalence of ED— ED is a highly prevalent and often underreported condition. The prevalence of ED varies in different countries (NIH Consensus Conference, 1993). Approximately 20-30 million men in the United States and approximately 0.5 million men in the UK have ED of varying severity (NIH Consensus Conference, 1993). According to the Massachusetts Male Aging Study, 52% of men in the United States between the ages of 40 and 70 years have ED (Feldman et al, 1994). The incidence is approximately 32% in the United Kingdom (Spector and Boyle, 1986), 26% in Japan (Melman and Gingell, 1999), and 19% in Denmark (Solstad and Davidsen, 1993). However, some men are unwilling to report the problem, because of their social and cultural backgrounds. This makes it difficult to determine the precise prevalence of ED. Furthermore, the paucity of epidemiological studies reported in the literature from developing and underdeveloped countries further compound this problem. Additional studies are needed to estimate the worldwide prevalence of ED. The available literature suggests that ED is a mounting problem.

Etiology of and Risk Factors for ED— The etiology of ED is multifactorial and can be classified as organic, neurogenic, and mixed. In organic ED, vasculogenic causes are common. The major risk factors implicated in the pathophysiological mechanism of organic ED are diabetes mellitus, hypercholesterolemia, smoking, and chronic medical illness. All of these factors increase the risk of atherosclerosis, which is the predominant predisposing cause of vasculogenic ED. Kaiser et al (1988) reported that atherosclerotic disease was the cause of ED in approximately 40% of men more than 50 years old (Kaiser et al, 1988). Atherosclerosis, along with other risk factors, is associated with endothelial dysfunction, which plays a crucial role in vasculogenic ED (Zeiher et al, 1993).

Physiological Mechanism of Erection— The physiological mechanism of erection is a complex neurovascular phenomenon that depends on neural, vascular, hormonal, and psychological factors. Integrated function of these factors is essential for production of a normal erectile response. Recent advances in the understanding of functional anatomy and of neurovascular interactions have improved our understanding of the pathophysiological mechanism of ED.

Role of NO Synthase (NOS) and NO in Erection. NO has been implicated in diverse physiological functions, including regulation of neural transmission in vascular tissue and immune system function (Moncada et al, 1991). The vasodilator effects in most vascular beds are mediated by endothelial derived relaxing factor (EDRF). NO has been established as an EDRF (Palmer et al, 1987) and shown to

play central role in the physiological mechanism of penile erection by initiating smooth-muscle relaxation (Burnett, 1997).

NOS.— Penile erectile tissue is formed by 2 dorsal corporal bodies known as the corpora cavernosa. The cavernosal bodies are composed of sinusoidal spaces with a trabecular meshwork. These spaces are lined by endothelium. Neural transmitters, such as acetylcholine, are released from cavernosal nerve endings and stimulate the neuronal NOS (nNOS) enzyme, which leads to the release of NO from the endothelium. Erectile function is mediated by both nNOS and endothelial NOS (eNOS) (Burnett et al, 1995). This was reported in studies involving transgenic mice lacking the gene encoding nNOS, which showed preserved erectile function, suggesting a possible alternative source of NOS from the endothelium (Huang et al, 1993). Later studies identified inducible NOS (iNOS), which also plays an important role in normal erectile response. The regulation of NOS is a complex phenomenon and is regulated by numerous factors. Inhibitors of inducible NOS (iNOS) that are more selective than those of eNOS and nNOS have been identified. Some of these have the potential to treat a range of inflammatory and noninflammatory conditions in which iNOS has been implicated (Alderton et al, 2001). Inflammatory cytokines, high levels of oxidized cholesterol, hypertension, and steroids down regulate the expression of eNOS (Maas et al, 2002).

NO in normal erection.— In the genitouriniary tract, NO is a neurotransmitter involved in nonadregenic, noncholinergic neurotransmission (Sanez de Tezada et al, 1989; Ignarro et al, 1990; Kim et al, 1991; Knispel et al, 1991; Andersson et al, 1992; Burnett et al, 1992; Rajfer et al, 1992; Burnett et al, 1993, 1995) and is a vasodilator produced by endothelial cells (Sanez de Tezada et al, 1989; Knispel et al, 1991). NO is an epithelial-derived factor (Burnett et al, 1995). It has been implicated in regional blood flow (Heaton et al, 1990; Holmquist et al, 1991; Burnett et al, 1992; Trigo-Rocha et al, 1993; Finberg et al, 1993), smooth-muscle function (Sanez de Tezada et al, 1989; Ignarro et al, 1990; Holmquist et al, 1991; Kim et al, 1991; Pickard et al, 1991; Burnett et al, 1992, 1993; Finberg et al, 1993; Trigo-Rocha et al, 1993; Burnett et al, 1995), and secretory responses in genitourinary tissues (Burnett et al, 1995).

NO is derived from the amino acid L-arginine via the L-arginine-NO pathway (Palmer et al, 1987). The synthesis of NO from L-arginine is catalyzed by the enzyme NOS (Palmer et al, 1988). In mammalian cells, L-arginine is used as a substrate by both NOS and arginase. In patients with uremia, arginine transport into the cell is inhibited, which could be a factor in ED in uremic patients (Xiao et al, 2001). Thus, arginase may down regulate NO production by competing with NOS for L-arginine (Bivalacqua et al, 2001). Arginase catalyzes the hydrolysis of L-arginine to form L-ornithine and urea, thus limiting the availability of L-arginine to form NO (Mori and Gotoh, 2000). Specifically, the L-arginine-NO pathway has been shown to play a significant role in mediating smooth-muscle relaxation of several nongenitourinary tissues (Calignano et al, 1992; Shuttleworth et al, 1993; Thirlby et al, 1993).

NO is the principal mediator of penile erection (Burnett et al, 1992). Erectile function is dependent on relaxation of the cavernous smooth muscle, and its mechanism of action is dependent on penile smooth-muscle relaxation mediated by NO. Immunohistochemical localization of NOS activity in the penile tissue provides confirmatory evidence that NO activity is important in erectile function (Burnett et al, 1992, 1993).

NO is synthesized by NOS. NO is a postganglionic neurotransmitter released from autonomic nerve terminals that diffuses into the vascular and cavernosal smooth muscle. In smooth muscle, NO activates guanyl cyclase and the increases cyclic guanosine monophosphate (cGMP) concentration. cGMP activates certain intracellular protein kinases that phosphorylate receptor proteins. Activated

protein kinases open the potassium channels and increase the influx of potassium (Seftel et al, 1996) and block the influx of calcium by inhibiting calcium channels (Figure 1). This leads to hyperpolarization and relaxation of smooth muscle. Reduced arteriolar resistance leads to sinusoidal spaces filled with blood. These enlarged sinusoids further increase the intracavernosal pressure by blocking the venous return and producing a rigid erection. cGMP is converted to 5 GMP by phosphodiesterase, which is inhibited by phosphodiesterase 5 (PDE-5) inhibitors.

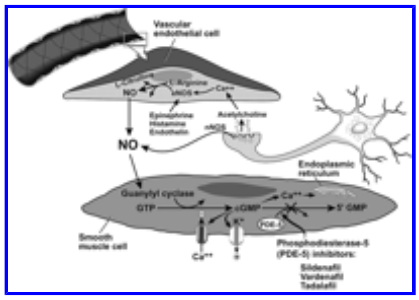

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Figure 1. Physiological mechanism of normal erection. Nitric oxide (NO) is synthesized from the endothelial cells by the enzyme NO synthase (NOS). It diffuses into the smooth muscle and stimulates guanyl cyclase. An increase in the cyclic GMP (cGMP) concentration stimulates the release of protein kinases. This causes the potassium channels to open and the calcium channels to close, producing hyperpolarization and, ultimately, smooth-muscle relaxation. eNOS, endothelial NOS; GTP, guanosine triphosphate; nNOS, neuronal NOS; PDE, phosphodiesterase enzyme.

The introduction of PDE-5 inhibitors (sildenafil, vardenafil, and tadalafil) changed the treatment algorithm for ED. Oral therapy has rapidly become the first treatment of choice for any form of ED. These drugs appear to increase the local NO effect by decreasing the destruction of cGMP. For unknown reasons, a certain percentage of the population does not respond to oral medication. Nonresponsiveness may be caused by reduced NO concentration due to endothelial dysfunction.

The precise pathophysiological mechanism of ED is still unclear. However, decreased production of NO or the absence of NO may play a major role. Production decreases when the availability of substrate for NOS is reduced. NO is a highly reactive free radical that undergoes nonenzymatic reaction with the heme moiety of oxyhemoglobin or that reacts with free radicals, such as superoxide anion, to form peroxynitrite (Beckman and Koppenol, 1996). This observation first highlighted the importance of oxidative stress in ED.

What Is Oxidative Stress?— Oxidative stress occurs when cells are exposed to excessive levels of reactive oxygen species (ROS) as a result of an imbalance between pro-oxidants and the protective mechanisms conferred by antioxidants (Zalba et al, 2000). ROS are formed during regular metabolism due to the univalent reduction of oxygen molecule. Superoxide (O_2^-) is the most important among the ROS. Hydrogen peroxide (H_2O_2), hypochlorous acid (HOCL), and peroxynitrite ($OONO^-$) are other important free radicals implicated in the pathophysiological mechanism of vascular disease. The vascular endothelium is the major source for these free radicals; platelets and leukocytes are the other important sources of ROS (Beckman and Koppenol, 1996).

Superoxide Radicals and Superoxide. Superoxide radicals are generated because of incomplete oxygen reduction in the electron transport system. Membrane-bound enzymes, such as nicotinamide adenine dinucleotide hydrogenase-nicotinamide adenine dinucleotide phosphate hydrogenase oxidase, are the major source of superoxide radicals in activated phagocytic cells (Kojda and Harrison, 1999). Several authors have reported that up regulation of these enzymes is associated with an increased risk of vascular disease (Warnholtz et al, 1999; Hink et al, 2001). Superoxide dismutase (SOD) is an important enzyme that removes the superoxide radicals from the human body. There are 3

types of SOD isoenzymes: cytosolic Cu Zn-SOD, mitochondrial Mn SOD, and extracellular SOD. Extracellular SOD reportedly plays a critical role in maintaining the redox state of vascular interstitium and thereby prevents the pathophysiological effects of superoxide in the vasculature. Extracellular SOD converts superoxide to H_2O_2.

Interaction Between NO and ROS: Hypothesis— The interaction between NO and ROS is one of the important mechanisms implicated in the pathophysiological process of ED (Jones et al, 2002). NO interacts with superoxide to form peroxynitrite, which has been reported to play a central role in atherogenesis (Beckman and Koppenol, 1996). Peroxynitrite reacts with the tyrosyl residue of proteins, which inactivates superoxide dismutase and leads to decreased removal of superoxide (Zou et al, 1997). This further increases the formation of peroxynitrite and reduces the available NO concentration. Peroxynitrite causes smooth-muscle relaxation and is less potent than NO. Khan et al (2001) studied the effect of NO and peroxynitrite on stripped cavernosal tissue from rabbits. They reported that relaxation induced by NO is short lived and immediate in onset, compared with that due to peroxynitrite, which is prolonged and slow in onset. Moreover, the tissues returned to original tension immediately with NO, whereas with peroxynitrite, the tissues were unable to recover their original tension. These mechanisms ultimately produce an ineffective relaxation in cavernosal tissue, which produces ED.

Peroxynitrite and superoxide have been reported to increase the incidence of apoptosis in the endothelium. This leads to denudation of endothelium and further reduction of available NO (Beckman and Koppenol, 1996; Khan et al, 2001). Superoxide is reported to have a direct vasoconstriction effect through mobilization of calcium ions (Katusic and Vanhoutte, 1989). This can potentially produce ED. According to the literature, the decreased availability of NO is the key pathophysiological process that leads to ED (Jeremy et al, 2000).

NO is reported to decrease the adhesion of platelets and leukocytes to the vascular endothelial cells (Figure 2). A reduced NO concentration aggravates the adhesion of these cells to the endothelium and releases substances (thromboxane A2 and leukotriens) that cause vasoconstriction. These substances further aggravate ED. The pioneering work conducted by Jeremy et al (2000) highlights the importance of oxidative stress in the endothelium and in ED and has opened a new era of interest. This also has lead many authors to evaluate the role of antioxidants in reducing oxidative stress.

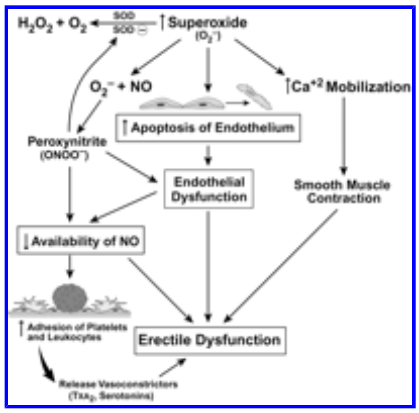

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Figure 2. Relationship between reactive oxygen species and erectile dysfunction, based on the hypothesis that superoxide radicals stimulate apoptosis, form peroxynitrite, and produce endothelial dysfunction. Peroxynitrite stimulates superoxide dismutase (SOD), produces ineffective smooth-muscle relaxation, and increases platelet adhesion to endothelium. Endothelial dysfunction and increased destruction of nitric oxide (NO) leads to erectile dysfunction. H_2O_2, hydrogen peroxide; $O_2^{\cdot-}$, superoxide free radical; TXA_2, thromboxane A2.

In contrast to the hypothesis discussed above, excessive production of NO has also been implicated as a possible cause of direct cavernosal damage. Excessive NO can be generated in the corpora cavernosa, especially in inflammatory conditions, such as Peyronie disease, penile trauma, and priapism. This increases the formation of peroxynitrite, leading to cytotoxic effects on cavernosal muscle (Wink et al, 1998). In vitro studies show that human cavernosal muscle exposed to high concentrations of NO significantly decreases DNA and ATP synthesis (Rajasekaran et al, 2001). Whether the cavernosal smooth muscle tissue is exposed to high levels of NO in vivo to induce cytotoxic effects is unclear. These studies suggest that increased peroxynitrite, secondary to increase NO availability, can produce cytotoxic effects.

Recently, low concentrations of oxidative stress were reported to have a more prominent proliferative effect on cavernosal smooth muscle than high concentrations, which inhibit cell growth (Sikka et al, 2005). Increased production of ROS (superoxide and peroxynitrite) reduces the effective NO concentration available for cavernosal muscle relaxation. The reduced availability of NO in acute disease and long-term endothelial damage are the 2 most important causes of ED.

Free radical damage is important in the aging process. Oxidative damage to the vasculature due to oxidative damage caused by superoxide anion plays a role in the natural aging process (Ames et al, 1993; Ferrara et al, 1995). The prevalence and severity of ED increase with age. The Massachusetts Male Aging Study reported that 52% of men between the ages of 40 and 70 years have some form of impotence. This study also revealed that aging is the most important factor associated with ED (Feldman et al, 1994). Aging is recognized to alter endothelial cell function, and the decrease in age-related erectile function has been attributed to reductions in NOS activity, impaired endothelial-dependent smooth-muscle relaxation, and diminished NO bioavailability (Garban et al, 1995; Carrier et al, 1997; Haas et al, 1998).

The process of aging is multifactorial in nature and dependent on several factors, including but not limited to metabolic rate, genetics, lifestyle, and environmental conditions (Schoneich, 1999), suggesting that aging is an independent risk factor. Whatever the initiating factor, the ultimate common pathological process is damage to smooth-muscle cells and an increase in the accumulation of fibrosis, which decrease the vasodilator response. This increased accumulation of collagen with aging has been observed in both human and rat corporal smooth muscle (Jevtich et al, 1990). The increase in collagen accumulation leads to a decrease in blood flow as measured by peak systolic velocity.

There is increasing interest among researchers to limit cavernosal fibrosis by use of various medications. Although there is no direct evidence supporting the role of vasodilator therapy in decreasing oxidative stress, reduced tissue hypoxia (which is an important factor in initiating oxidative stress) might be a factor in decreasing oxidative stress (Sommer and Englemann, 2004). These investigators studied the effects of daily sildenafil in 76 patients who had ED for at least 6 months. The patients were randomly assigned to 1 of the following 3 groups: group 1 received 50 mg of sildenafil every night at bedtime, group 2 received 50-100 mg of sildenafil on demand, and group 3 did not receive treatment. After a 12-month follow-up period, group 1 contained a larger number of patients with normal erectile function and peak systolic velocity. The authors concluded that daily sildenafil taken at bedtime might cure ED in the long-term by improving penile blood flow. Aging not only increases the prevalence of ED but also increases the severity. Although the development of ED is multifactorial in nature, it is typically associated with vascular diseases and risk factors, such as artherosclerosis, hypertension, diabetes mellitus, and cigarette smoking (Feldman et al,

1994; Laumann et al, 1999; Bivalacqua et al, 2000).

Role of Risk Factors in ED: Interaction With ROS

Population-based studies show that diabetes, hypertension, and cigarette smoking are known independent risk factors for ED (Mannino et al, 1994; Burchardt et al, 2000; Martin-Morales et al, 2001). The risk of ED also appears to be increased for patients with hypercholesterolemia (Wei et al, 1994). These factors increase the risk of atherosclerosis. Atherosclerosis is associated with diminished bioavailability of NO, which has been widely believed to be one of the most important causes of cardiovascular disease. Patients with risk factors, such as hypertension, diabetes, hypercholesterolemia, and hyperhomocysteinemia, appear to have a decreased availability of NO, because of increased generation of ROS.

Diabetes— ED is more common among men with diabetes mellitus than in the general population. Approximately 75% of men with diabetes have ED, which occurs at an early age (Newman and Marcus, 1985). Impaired cavernosal smooth-muscle relaxation mediated by endothelial and neuronal mechanisms has been observed in human and animal diabetic models (Saenz de Tejada et al, 1989). Oxidative stress-mediated neurovascular alteration appears to play an integral role in the development of ED in the diabetic population.

Impaired NO activity in diabetic volunteers was first reported in 1989 (Saenz de Tejada et al, 1989). However, Escrig et al (2002) recently reported that diabetes-induced reduction in corporeal NO levels could be mainly due to the lack of essential cofactors for NOS activity. This was further supported by the observation that cavernosal smooth-muscle relaxations are restored by long-term administration of L-arginine (a substrate for NOS) (Gur et al, 2000). However, studies involving the systemic and cavernosal vasculature demonstrated that superoxide-induced inactivation, rather than decreased activity of NOS, is the major cause of reduced NO levels (Beckman and Koppenol, 1996; Laight et al, 2000).

In a similar experiment conducted by Ryu et al (2003) in rats with streptozotocin-induced diabetes, the authors observed that the mean intracavernosal NO pressure (±SD) was significantly lower in the rats with long-term diabetes (49.8 ± 9.4 cm H_2O) than in the rats with short-term diabetes (75.9 ± 14.8 cm H_2O). The pressure was profoundly lower in diabetic rats, compared with age-matched controls (Ryu et al, 2003). Furthermore, glutathione levels were also significantly lower in diabetic animals than in age-matched controls. These observations further strengthen the relationship between oxidative stress and ED in diabetic patients and provide indirect evidence that the duration of diabetes increases the risk of ED.

The role of superoxide in diabetic animals is further supported by the findings of Jeremy et al (2000). They reported that superoxide production is markedly elevated in the cavernosum of rats with streptozotocin-induced diabetes. Pretreatment with SOD in diabetic rat aortas produced a greater degree of relaxation than no pretreatment in the controls (Langenstroer and Pieper, 1992). This finding was further supported by the observation that probucol, an antioxidant, prevented the diabetes-induced impairment of endothelial-dependent relaxations in aortic rings. These studies suggest that an increased superoxide concentration might play a key role in cavernosal smooth-muscle dysfunction.

Two other mechanisms have been implicated in diabetic ED: activation of protein kinase C and formation of advanced glycation products. Protein kinase C is an enzyme that modulates several cellular events, and increased levels of this enzyme are associated with increased production of ROS

and altered levels of NO, which can be prevented by administration of antioxidants (Ganz and Seftel, 2000). Advanced glycation products quench NO, and their production is associated with increased superoxide production (Mullarkey et al, 1990).

O-linked N-acetylglucosamine (O-GlcNAc) is the major product of advanced glycation implicated in cavernosal dysfunction in diabetic patients. Recently, Burnett et al (2005) investigated whether hyperglycemia increases the O-GlcNAc-induced modification of eNOS in rats with diabetes. They reported a significant increase in the modification of eNOS and the reduced phosphorylation of eNOS by O-GlcNAc at baseline and after electrical stimulation in diabetic rats, compared with controls. They also reported that the penis in rats with diabetes had a significantly decreased response to electrical stimulation. This study suggests that a specific glycation mechanism impairs eNOS function in diabetic animals (Burnett et al, 2005). It is evident from initial studies conducted using animal models that increased superoxide or advanced glycation products decrease NO availability in cavernosal smooth muscle, which ultimately might impair smooth-muscle relaxation.

Hyperhomocysteinemia— Elevated levels of homocysteine (an amino acid) or hyperhomocysteinemia are associated with increased superoxide production, which results in endothelial dysfunction. This phenomenon has been implicated in the development of atherosclerosis and impaired relaxation of vascular tissue, which predisposes humans to cardiovascular disease (Boushey et al, 1995; McDowell and Lang, 2000), and has been proposed to exert a similar effect in cavernosal smooth muscle in animal models. Elevation of homocysteine levels was associated with increased production of superoxide levels and significant reduction in cGMP levels (Jones et al, 2005). The precise mechanism of superoxide formation in patients with homocysteinemia is still unclear. However, this study suggests that oxidative stress may be a cause of ED in patients with hyperhomocysteinemia.

Hypercholesterolemia— Hypercholesterolemia is associated with increased ultrastructural predisposition to atherosclerosis and decreased cavernosal smooth-muscle relaxation (Kim et al, 1994). Normalization of cholesterol levels enhances cavernosal smooth-muscle relaxation (Kim et al, 1994). Cavernosal tissue superoxide levels are elevated in rabbits that received 2% cholesterol for 2 months (Kim et al, 1997). Increased cavernosal superoxide levels in hypercholesterolemia may decrease the availability of NO, which may lead to ED. The effect of hypercholesterolemia on human cavernosal smooth muscle was not documented in experimental models. However, further studies are essential to establish the exact role of hypercholesterolemia in ED and its correlation with oxidative stress.

Hypertension— Hypertension is one of the important risk factors for atherosclerosis-induced vascular damage (Feldman et al, 1994). This vascular damage is a major cause of ED in hypertensive patients. However, alterations in the neurogenic pathway may also play a role in hypertension (Ushiyama et al, 2004). NO has been the principle vasodilator involved in cavernosal smooth-muscle relaxation. However, there is some evidence suggesting that alternative neurotransmitters, such as carbon monoxide (CO), also have vasodilator properties (Tenhunen et al, 1969), and impaired CO-mediated vasodilatation has been implicated in ED. Ushiyama et al (2004) first reported the role of CO and NO in inducing ED in hypertensive rats. They compared cavernosal smooth-muscle relaxation after electrical stimulation in spontaneous hypertensive rats with that in normotensive rats. The CO-mediated and NO-mediated relaxations were significantly impaired in the hypertensive rats and were associated with reduced activity of superoxide dismutase.

Spontaneously hypertensive rats have a higher blood pressure and excrete greater levels of 8-isoprostaglandin F2α (which is formed nonenzymatically from the attack of superoxide radical on arachidonic acid) than do normotensive rats. In humans, a significant correlation is seen between

mean blood pressure and oxidative stress in polymorphonuclear leukocytes (Yasunari et al, 2002). Data point to the role of increased concentrations of reactive oxygen species and vasoconstriction in patients with essential hypertension due to endothelial dysfunction and/or reduced vasodilator activity (Taddei et al, 2001). These findings support the hypothesis that ED in hypertensive rats may result from impaired relaxation induced by neurogenic CO and NO.

Hypertension is also a common complication of chronic renal failure (Himmelfarb et al, 2002) and is frequently associated with end-stage renal disease. Several studies have revealed evidence of increased oxidative stress in chronic renal failure and pathogenesis of hypertension (Swei et al, 1997). Several studies have shown that certain forms of genetic or acquired hypertension are associated with oxidative stress (Gonick et al, 1997; Vaziri et al, 1997; Ding et al, 1998; Vaziri et al, 1999, 2000; Ding et al, 2001). Deficiency of endothelial NO has been implicated in some forms of hypertension (Huang et al, 1995; Baylis and Vallance, 1996; Soma et al, 1999). Increased concentrations of reactive oxygen species (Tepel et al, 2000), elevated levels of plasma lipid oxidation products, increased levels of F2-isoprostanes, increased myeloperoxidase catalyzed oxidation, and reduced levels of antioxidant enzymes have been reported in chronic renal failure (Himmelfarb et al, 2002).

An increase in the angiotension II level is one of the factors responsible for an increase in blood pressure and for maintenance of hypertension. Elevated angiotensin levels have also been reported to be associated with increased oxidative stress (Ruiz-Ortega and Ortiz, 2005). These effects could be mediated by a reduction in the concentration of NO, along with the formation of F2-isoprostanes and endothelin (Romero and Reckelhoff, 1999; Reckelhoff and Romero, 2003). Decreased NO bioavailability in obesity-prone animals has been shown to be due, in part, to increased oxidative stress (Dobrian et al, 2001; 2003). Increased superoxide production in both the vasculature and the kidney has been extensively reported in various forms of hypertension in experimental models and in humans (Sagar et al, 1992; Swei et al, 1997; Russo et al, 1998; Schnackenberg et al, 1998).

Hypertension is one of the most widely prevalent diseases. A significant proportion of the hypertensive population has ED. It is essential to identify the measures to reduce the incidence ED in this population. The role of antioxidants in reducing the prevalence of ED needs to be established in further human trials.

Treatment Options Available for Oxidative Stress

Role of Antioxidants— Antioxidant defenses limit the indiscriminate damage caused by oxygen free radicals. Antioxidants can be endogenous or exogenous. Antioxidant enzymes (eg, SOD and catalase), chain-breaking antioxidants (eg, vitamin E, carotenoids, and flavonoids), and transitional metal binding proteins (eg, ferritin) are the 3 basic types of antioxidants that defend against free radicals. The role of antioxidants, vitamin E in particular, has been extensively investigated in animal models in the prevention of atherosclerosis.

Vitamin E. Vitamin E is an important antioxidant that helps prevent lipid peroxidation (Esterbauer et al, 1991). It reacts more rapidly than polyunsaturated fatty acids with peroxyl radicals. Thus, it is known as a lipidphase, chain-breaking antioxidant. It has been shown to enhance endothelial function by increasing free radical trapping. In vivo studies revealed that vitamin E improved NO-mediated arterial relaxation. Keegan et al (1995) evaluated the role of vitamin E in the prevention of defective endothelium-dependent relaxations in the diabetic rat aorta and reported that exogenously administered vitamin E significantly attenuated diabetes-induced defective relaxations. This study highlighted the role of vitamin E in scavenging excessive reactive oxygen species. Other studies suggest that vitamin E inhibits monocyte adhesion and cytokine release

(Islam et al, 1998) and platelet adhesion and aggregation by a protein C kinase-dependent mechanism (Islam et al, 1998; Saldeen et al, 1999). This protein C kinase-dependent mechanism has been used to limit glucose-induced vascular dysfunction in patients with diabetes mellitus. Both in vivo and in vitro evidence suggest that the administration of a tocopherol can reverse protein C kinase activation and prevent endothelial dysfunction.

Vitamin E and efficacy of PDE-5 inhibitors.— The introduction of PDE-5 inhibitors has been one of the major advances in the treatment of ED. Their use has significantly changed the algorithm for treatment of ED. Because of increased patient compliance, these drugs are now used as first-line agents in the treatment of ED. However, they are effective only in 74%-97% of the general population and in 50%-56% of the diabetic population (Rendell et al, 1999). The precise reasons for this lack of response are unclear, although many authors believe that free radical-induced decreases in the NO concentration may be a cause.

Circulating levels of NO may be enhanced through the delivery of adequate concentrations of free radical scavengers, such as vitamin E. Higher levels of NO, therefore, should theoretically increase penile blood flow, with the potential for a synergistic effect when combined with a PDE5 inhibitor. This hypothesis was recently validated in an animal model (De Young et al, 2003). In this study, 20 adult male Sprague-Dawley rats with streptozotocin-induced diabetes were divided into the following 4 treatment groups: group 1 (the control group) received no treatment, group 2 received 20 IU/d of vitamin E, group 3 received 5 mg/kg/d of sildenafil, and group 4 received 20 IU/d of vitamin E and 5 mg/kg/d of sildenafil. The treatments were given for 3 weeks through oral gavage. Erectile function was assessed as an increase in intracavernous pressure after an interval of 16-20 hours. A significant increase in intracavernous pressure was observed in the animals receiving combined treatment with vitamin E and sildenafil. Immunohistochemical staining of the cavernosal tissue revealed an increase in the nNOS level, endothelial cell staining, and smooth-muscle cell staining. Vitamin E enhanced the therapeutic effect of the PDE-5 inhibitor in the animal model of diabetes. This study suggests a potential way of salvaging erectile function for patients who are refractory to sildenafil therapy.

In 2004, the same authors reported results of a comparison of cavernosal pressure between 20 diabetic rats and 5 nondiabetic rats (De Young et al, 2004). Twenty rats with streptozotocin-induced diabetes were divided into the following 4 treatment groups: group 1 (the control group) received no treatment, group 2 received 20 IU/d of vitamin E, group 3 received 5 mg/kg/d of sildenafil, and group 4 received 20 IU/d of vitamin E and 5 mg/kg/d of sildenafil. These treatments were also administered through oral gavage. Penile tissue was evaluated for nNOS level, smooth-muscle α-actin level, nitrotyrosine level, and endothelial cell integrity. Urine nitrite and nitrate concentrations were quantified, and electrolyte levels were measured. After a 3-month follow-up period, a significant difference in cavernosal pressure was observed between the control group and the groups receiving combination therapies. Urine nitrite and nitrate concentrations increased significantly in all diabetic groups but decreased in the vitamin E group and the vitamin E plus sildenafil group. A significant increase in the nitrotryosine level was observed in the vitamin E plus sildenafil group. Positive results of immunochemical staining revealed that vitamin E enhanced the therapeutic effect of the PDE-5 inhibitor, supporting the potential use of antioxidants in salvaging erectile function in diabetic patients. The results of these animal studies need to be confirmed in human trials with enrollment based on use of validated questionnaires before they can be used in routine clinical practice.

Other antioxidants. The role of antioxidants, such as a-lipoic acid (ALA) and linolenic acid, in the prevention of cavernosal dysfunction was examined in vivo in a diabetic rat model (Keegan et

al, 1999). ALA has both free radical and metal chelator properties. Administration of ALA was associated with preservation of nonadrenergic and noncholinergic cavernosal relaxation in diabetic animal models. γ-Linolenic acid, when combined with ALA, produced a similar degree of cavernosal relaxation at lower ALA concentrations. Diabetes reduces endothelium-mediated and nonadrenergic, noncholinergic nerve NO-mediated relaxation of corpus cavernosum smooth muscle, which is likely to be the organic base for impotence. Prevention and partial correction of cavernosal dysfunction by ALA emphasizes the importance of reactive oxygen species and suggests a potential therapeutic approach. However, the role of these antioxidants needs to be further quantified in both animal and human experiments before they can be used in humans.

Future Directions

NOS Donors— Recently, the area of interest in ED research has shifted to NO donors—drugs that increase NO synthesis in the cavernosal bodies. Fillippi et al (2003) investigated the effects of NCX 4050 (a drug belonging to a new class of NO donors) on isolated preparations of human and rabbit corpus cavernosa. They reported that NCX 4050 increased guanyl cyclase activity and resulted in smooth-muscle relaxation in both human and rabbit cavernosal models (Filippi et al, 2003). Recently, Kalsi et al (2004) reported that NCX 911, a NO-releasing PDE-5 inhibitor, induced the relaxation of cavernosal smooth muscle by increasing the concentration of endogenous NO. These 2 agents may be promising future treatment options for patients with impaired NO release from the endothelium. However, the efficacy of these agents needs to be confirmed in humans.

Extracellular Superoxide Dismutase: Gene Transfer— Superoxide is the key reactive oxygen species involved in the pathophysiological mechanism of ED. Superoxide dismutase plays an important role in conferring protection against O_2^- radicals. Superoxide is one of the most important free radicals implicated in the pathophysiological mechanism of vascular dysfunction observed in hypertension, atherosclerosis, and diabetes mellitus. It represents a major cellular defense against superoxide and peroxynitrite formation. Of the 3 superoxide dismutases noted above, extracelluar SOD is hypothesized to play a critical role in modulating the redox state of the vascular interstitium, thereby preventing the pathophysiological effects of superoxide in the vasculature.

The application of gene transfer techniques involving some basic modulators of penile erection to improve erectile response in animal models has opened up new directions for gene therapy in the field of erection physiology (Champion et al, 1999; Bivalacqua et al, 2003). Investigators have used an adenovirus as a vector for the gene encoding eNOS to assess whether the gene can be overexpressed in the aged rat penis to improve erectile responses. They also reported that biochemical analysis revealed that the adenovirus vector transferred the eNOS successfully to the aged rat corpus cavernosum and that the gene encoding eNOS was functionally relevant in increasing intracavernosal pressure in response to pharmacological and electrical nerve stimulation in vivo.

These results provide important support for the functional relevance of eNOS in the regulation of erectile function, and they establish that in vivo transfer of the gene encoding eNOS could constitute a meaningful therapeutic intervention for the treatment of ED. This successful application of gene therapy is pertinent for similar future strategies involving multiple possible regulatory factors associated with penile erection. There is a growing interest in gene transfer of extracellular SOD through viral vectors (Bivalacqua et al, 2003). Bivalacqua et al (2003) transfected rats with viral vectors containing the gene encoding extracelluar SOD. This resulted in increased expression of extracellular SOD mRNA, SOD activity, and cyclic guanosine monophosphate levels. In their in vivo study, Bivalacqua et al (2003) demonstrated that transfection with viral vectors results in a significant increase in erectile response to cavernosal nerve stimulation. The

advantage appeared to be more prominent in aged rats, indicating that cavernosal dysfunction associated with aging is related, in part, to an increase in superoxide formation. This landmark study highlighted the use of a novel technique in restoring erectile function. Gene transfer might serve as an important future therapeutic option in the treatment of ED, especially in the aged population. However, the safety and applicability of these vectors needs to be tested in human studies.

Limitations of Existing Animal Model Studies

The relationship between reactive oxygen species and ED was largely derived from in vitro studies investigating animal models (Table). It is difficult to draw specific conclusions from these studies, as the results are not exactly reproducible in humans. Moreover, the concentration of various constituents to which the experimental tissues are exposed may not be identical to that in vivo. This holds true particularly for studies evaluating the role of medications. It is difficult to predict the dose of the medication that needs to be administered in humans to produce similar results (De Young et al, 2003, 2004). It is not possible to directly measure the NO concentration because of its short half-life, which means that we must depend on indirect measurements, such as the degree of cavernosal smooth-muscle relaxation. This has further increased the ambiguity of the results of such studies. However, these studies have highlighted the role of oxidative stress in ED and have added a new dimension to the existing literature, which, until now, mainly focused on the involvement of neurotransmitters.

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Studies showing the relationship between oxidative stress and erectile dysfunction[*]

Conclusions

Normal penile erection is dependent on the integrity of the endothelium. Endothelial-derived NO plays an important role in the physiological mechanism of erection. Alteration in the concentration of NO, due to damage to endothelium or to increased destruction, appears to be the most important causes for ED, especially in the presence of vascular disease. In vitro studies showed that increased production of reactive oxygen species is associated with decreased normal erectile response, primarily because of reduced NO concentrations. Increased production of reactive oxygen species in diseases such as diabetes and hypertension might be an important cause of an increased risk of ED. Novel strategies and interventions are needed to prevent and treat endothelial damage.

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