Chinese Journal of Physiology 49(3): 160-166, 2006

Changes of Nitric Oxide Synthase-Containing Nerve Fibers and Parameters for Oxidative Stress after Unilateral Cavernous Nerve Resection or Manuplation in Rat Penis

Hamdi Özkara[1], Cabir Alan[1], Pinar Atukeren[2], Ilhan Uyaner[3], Cihan Demirci[3], M. Koray Gümüştaş[2], and Bulent Alici[1]

[1]*Cerrahpasa Medical Faculty*
Department of Urology, Istanbul University,
[2]*Cerrahpasa Medical Faculty*
Department of Biochemistry, Istanbul University,
and
[3]*Science Faculty*
Department of Biology, Istanbul University,
Istanbul, Turkey

Abstract

After pelvic surgeries such as radical prostatectomy, two major complications – urinary incontinence and erectile dysfunction (ED) may occur. Etiologies for ED are multiple pathologic mediators/systems. Oxidative stress, which is known to be induced after surgical trauma, could be a cause of ED. The purposes of in this study are to investigate the effect of unilateral manipulation/ dissection and resection of the cavernous nerve (neurotomy) to NOS (nitric oxide synthase)-containing nerve fibers and pressure after electro stimulation in rat corpus cavernosum, and to determine whether these procedures would produce oxidative stress within rat cavernous tissue 3 weeks and 6 months after the operation. Male rats were divided into 5 groups. Rats in groups 1 and 2 underwent unilateral cavernous nerve manipulation and sacrificed 3 weeks and 6 months after the operation, respectively. Rats in groups 3 and 4 underwent unilateral neurotomy of a 5-mm. segment of the cavernous nerve, and they were sacrificed 3 weeks and 6 months after nerve ablation, respectively. Group 5 rats were control animals for biochemical analysis. Intracavernous pressure following electro stimulation reduced is significantly 3 weeks after unilateral resection, as compared to that of the manipulated nerve ($P < 0.05$), and it recovered 6 months after neurotomy. The recovery was also confirmed by NADPH (nicotinamide adenine dinucleotide phosphate) diaphorase staining in neurotomy groups. Lipid peroxidation, which is an indicater of oxidative stress, was determined by measuring thiobarbituric acid reacting substance (TBARS) levels and superoxide dismutase (SOD) activity. These markers indicated that unilateral cavernous nerve manipulation or resection produced oxidative stress within rat corpus cavernosum. Oxidative stress was more prominent 3 weeks after unilateral neurotomy ($P < 0.05$). Also, compared to the control animal group, oxidative stress was observed three weeks after manipulation of unilateral cavernous nerve ($P < 0.05$). Resection of the cavernous nerve caused more prominent oxidative stress than in the manipulation group. This study suggested, that unilateral cavernous neurotomy caused a decrease of intra cavernous pressure and NOS fibers in rat corpus cavernosum, and they recovered 6 months after neurotomy. Our data also provided evidence that neurotomy and manipulation of the cavernous nerve caused oxidative stress in rat corpus cavernosum and that oxidative stress was more prominent in the nerve resection group.

Key Words: nitric oxide, nitric oxide synthase, neurotomy, erectile dysfunction, oxidative stress

Corresponding author: Dr. M. Koray Gumustas, Istanbul University, Cerrahpasa Medical Faculty , Department of Biochemistry, 34303, Cerrahpasa, Istanbul-Turkey. Tel: 00-90-212-414 35 84, E-mail: profgumustas@yahoo.com
Received: January 26, 2005; Revised: April 27, 2005; Accepted: May 5, 2005.

Introduction

Radical pelvic surgery like radical prostatectomy for prostate cancer is widely performed nowadays, and its major complication is the loss of erectile function. A better understanding of the penile neuroanatomy has enabled surgeons to perform nerve-sparing surgery. Although nerve-sparing prostatectomy significantly reduces postoperative erectile dysfunction, a large number of patients still have inadequate penile rigidity. Previous studies have reported the recovery of erectile function in 41-69% of men in whom at least one neurovascular bundle is preserved, in the 6- to 18-month postoperative period (6, 20). Erectile dysfunction (ED) may also occur after bilateral cavernous nerve preserving (16). The pathogenesis of erectile dysfunction, as a complication following nerve sparing pelvic surgery, awaits further investigation.

Many *in vivo* and *in vitro* studies have identified the nonadrenergic noncholinergic (NANC) neurotransmitter as the principal pathway of penile erection, and nitric oxide (NO) is the main neurotransmitter (4). However, because of the gaseous nature and short half-life of NO, its presence may only be studied indirectly. Thus, staining of the enzyme NO synthase (NOS) which NO from L-arginine has been used to recognize and quantify NOS-containing nerves in the penis. Nicotineamide adenine dinucleotide phosphate (NADPH) diaphorase specifically stains at the same site as a specific neuronal NOS-antibody, and neuronal NADPH diaphorase is a nitric oxide synthase. NADPH diaphorase histochemistry, therefore, provides a specific histochemical marker for neurons producing nitric oxide (9).

Oxidative stress has been addressed recently as an etiological factor for ED in diabetic rats (18). Surgical traumas are also considered to be a cause for oxidative stress (14). Oxidative stress results from an imbalance between the formation of free radicals in the tissue and their scavengers, and causes accumulation of reactive oxygen species (ROS) in the tissue. ROS, which can accumulate in the corpus cavernosum during oxidative stress, are shown to be a possible cause for ED (18). Oxidative stress has been reported as one of the major determinants of NO breakdown (12). If nerve manipulation or resection during pelvic surgery causes oxidative stress within the corpus cavernosum, it may be a possible reason for ED after pelvic surgical trauma. Oxidative stress could be determined in experimental studies by lipid peroxidation (Lpx) and superoxide dismutase (SOD) activity in the tissue. In this study, we aim to discover the effect of unilateral dissection (manipulation) or resection of cavernous nerve to NOS-containing nerve fibers and pressure after electro stimulation in the rat corpus cavernosum, and whether these procedures would produce oxidative stress within rat cavernous tissues after 3 weeks and after 6 months.

Materials and Methods

Experiments were performed on 46 adult fertile male Sprague-Dawley rats (aged 60-90 days, 250-300 gm.), divided into 5 groups: group 1 = 9 animals sacrificed 3 weeks after unilateral (left) cavernous nerve manipulation; group 2 = 9 animals sacrificed 6 months after unilateral (left) cavernous nerve manipulation; group 3 = 9 animals sacrificed 3 weeks after unilateral (left) cavernous nerve resection; group 4 = 9 animals sacrificed 6 months after unilateral (left) cavernous nerve resection; and a control group (n = 10). Rats in the control group were used for biochemical analysis, and they did not undergo any surgical procedure before sacrification (so that we could tell the TBARS and SOD levels in rat corpus cavernosum without any surgical procedure).

Surgical Procedure

The rats were anesthetized with pentobarbital sodium (35 mg/kg) intraperitoneally. Operations were performed with an operation-loop (Heine, Germany, 4.5X). Through a lower abdominal midline incision, the area posterolateral to the prostate was explored on one side (left) and the pelvic plexus, pelvic nerve and cavernous nerve were identified and exposed. In the manipulation groups (groups 1 & 2), the cavernous nerve was dissected from underlying prostate tissue but not cut. In the neurotomy group (groups 3 & 4), unilateral cavernous nerve ablation was performed by removing a segment as described previously (5). The wound was closed in a two-layer fashion with running suture of absorbable 3-0 Dexon.

Functional Evaluation and Tissue Procurement

Before sacrificing, the rats (excluding the control group) underwent exploration for direct electro stimulation of the contralateral cavernous nerves (right) before tissue collection. After exposing pelvic organs and cavernous nerve, the ischiocavernous muscle was partly removed to expose the crus of the penis. A 23-gauge butterfly needle was inserted into the crus penis for intra cavernous pressure measurement. Electro stimulation was performed with a slight stainless steel bipolar hook electrode. Each pole was 0.1 mm in diameter, and the two poles were separated by ~1 mm. A custom-built constant current amplifier generated electro stimulation. Stimulus parameters were 1.5 mA, frequency 20 Hz, pulse width 0.2 msec, for the duration of 1 min. Intra cavernous pressure was measured by a pressure transducer (DTX Plus

TNF-R, Becton Dickinson, Singapore) and recorded by cavernous pressure recorder (Cavomat 7000, Wiest, Germany). After functional evaluation, the ~5 mm segment of the crus penis distal to needle insertion was excised and divided transversally into two pieces. One part was put into fixative for NADPH-diaphorase staining and the other part embedded into liquid nitrogen immediately for biochemical assays. Needle insertion and electrostimulation did not effect on the subsequent NADPH-diaphorase staining, in the literature penile shaft after functional evaluation was also used for cavernous tissue staining (5-11). Tissue procurement of the control group for biochemical analysis was completed as described without electrostimulation.

NADPH-diaphorase Staining

Pieces taken from the penis were fixed in 20% para formaldehyde. For histochemical staining, sections in 0.008 mm were taken on glass slides, and NADPH-diaphorase histochemistry was applied to these cryostat sections. Slides were incubated in 0.1 mol phosphate buffer containing 0.3% Triton X-100, 0.01% nitroblue tetrazolium (NBT), and 0.1% β-NADPH for 25-30 min. The catalytic activity of NOS was demonstrated by enzymatic reduction of NBT in the presence of NADPH (NADPH-diaphorase reaction).

The presence of NADPH diaphorase-positive structures was determinated as blue dense regions by staining. The staining pattern was assessed by counting the number of NADPH diaphorase-positive regions (blue dense regions) within each corpus cavernosum in two arbitrary areas (magnification X 400). The endothelium staining was not included in the count.

Tissue Homogenization

The tissue samples were weighed (wet weight) and homogenized in ice-cold NaCl 0.9% to produce a 1/10 (w/v) homogenate. Tissue homogenization was performed with a tissue grinder fitted with a Teflon pestle. The homogenate was sonicated with MSE sonicator two times at 30 sec intervals on ice, with a power output of 38 watts. The sonicated homogenates were centrifuged at 2000 g for 10 min for the measurement of lipid peroxidation and 15000 g for 15 min for the measurement of the superoxide dismutase (SOD) activity, at 4°C. The biochemical assays were performed in the supernatants.

Measurement of the Lipid Peroxidation

Tissue TBARS (Thiobarbituric acid reacting substances) levels, as the marker of lipid peroxidation was determined with the spectrophotometric method described by Buege and Aust (3). Stock solution: 15% trichloroacetic acid (TCA), 0.375% thiobarbituric acid (TBA), and 0.25 N hydrochloric acid (HCl). The samples were heated in a water bath for 20 min and after cooling, centrifuged at 2000 g for 15 min and the formation of a pink color as a result of the reaction in between one molecule of TBARS and two molecules of thiobarbituric acid (TBA), is measured at 560 nm spectrophotometrically.

Measurement of the Tissue SOD Activity

Tissue SOD activity was measured by the modified method of Sun; the activity of SOD was measured in an alkaline medium that is titrated to pH: 10.2 during the experiment (19). This assay for superoxide dismutase (SOD, EC 1.15.1.1) activity involves inhibition of nitro blue tetrazolium (NBT) reduction, with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Reaction mixture consisted of 40 ml of 0.3 mM xanthine solution, 20 ml of 0.6 mM EDTA solution, 20 ml of 150 μM nitro blue tetrazolium solutions, 12 ml of 400 mM Na_2CO_3 solution and 6 ml of bovine serum albumin. The final concentration of xanthine oxidase was then 167 U/l. The production of formazon was determined spectrophotometrically at 560 nm.

Statistical Analysis

Results were compared with one-way ANOVA. Tukey HSD (Honestly Significant Difference) tests were used for multiple comparisons. Values were considered significant at $P < 0.05$. Data are expressed as at the mean ± standard deviation (S.D.).

Results

Electro stimulation of the cavernous nerve revealed in the mean maximum intracavernous pressure of nerve manipulation groups (groups 1 & 2) 80.55 ± 9.82 and 87.22 ± 9.05 cm H_2O, respectively. There was no difference between groups 1 and 2 ($P > 0.05$). Electro stimulation of the intact cavernous nerve of groups 3 and 4 showed in the mean maximum intracavernous pressure 33.89 ±16.91 and 58.88 ± 12.94 cm H_2O, respectively (Table 1). Intracavernous pressure of group 4 was significantly increased, as compared to group 3 ($P < 0.05$), but it was still lower than groups 1 and 2 ($P < 0.05$).

Histological examination of penile specimens revealed a separate staining pattern in the corpora

Table 1. Intracavernous pressure after electro stimulation

	Intracavernous pressure
Group 1	80.55 ± 9.82
Group 2	87.22 ± 9.05
Group 3	33.89 ± 16.91*
Group 4	58.88 ± 12.94**

Values represent mean ± S.D. maximal intracavernous pressure (cm H_2O)
One-way ANOVA test; F = 33.05, $P < 0.0001$
Post-huc Tukey HSD test were used for multiple comparisons.
*$P < 0.05$ versus all
**$P < 0.05$ versus all

Table 2. Presence of NADPH diaphorase positive nerve fibers in cavernosal tissue

	NADPH diaphorase (+) staining
Control	105.56 ± 14.24
Group 1	92.22 ± 9.71
Group 2	98.89 ± 12.69
Group 3	17.22 ± 6.67*
Group 4	48.89 ± 19.00**

Each value represents mean ± S.D. number of NADPH diaphorase-positive staining regions within each corpus cavernosum in 2 arbitrary areas (magnification × 400)
One-way ANOVA test; F = 74.41, $P < 0.0001$
Post-huc Tukey HSD test were used for multiple comparisons.
*$P < 0.05$ versus all
**$P < 0.05$ versus all

cavernosa of all groups. The number of NOS-containing nerve fibers within corpus cavernosum was significantly decreased in both groups 3 and 4 compared with groups 1 and 2 (Table 2). The NADPH diaphorase staining of group 3 was significantly worse, as (Fig. 1a) compared to group 4 (Figure 1b) (17.22 ± 6.67 and 48.89 ± 19.00 respectively) ($P < 0.05$). There were no differences between the staining of groups 1 and 2 (92.22 ± 9.71 and 98.89 ± 12.69, respectively) ($P < 0.05$).

The level of lipid peroxidation (Lpx) of corpora cavernosa was determined by measuring TBARS (Thiobarbituric acid reacting substances) levels of wet tissue. Tissue TBARS level was found significantly lower in the control group (28.64 ± 3.97 nmol/gr wet tissue), as compared to other groups ($P < 0.05$ versus all; Fig. 2). The result of group 3 (79.11 ± 8.42) was significantly higher than those of the other groups ($P < 0.05$ versus all). The TBARS level of group 4 (59.13 ± 6.68) is higher than those of the groups 1 and 2 (46.37 ± 3.09 and 40.65 ± 3.75, respectively) ($P < 0.05$).

SOD activity of all surgical procedure-applied animals (groups 1, 2, 3, 4) was significantly lower than the control group ($P < 0.05$) (Fig. 3). In group 2,

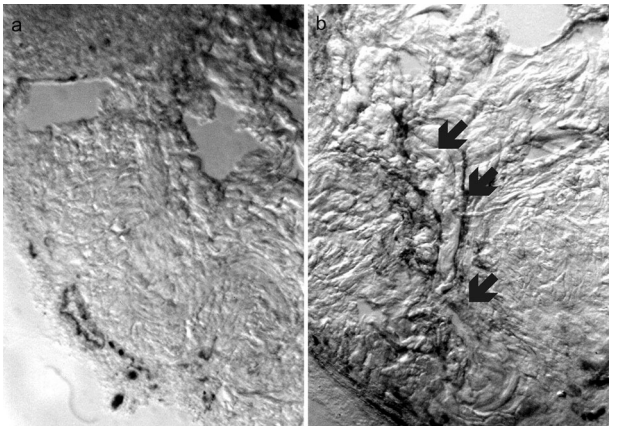

Fig. 1. NADPH-diaphorase staining of unilateral cavernous nerve ablation indicated that at 3 weeks, there was absence of NADPH- diaphorase staining of cavernous tissue (a); at 6 months, minimum number of NOS-containing fibers as dark dense regions (arrows) are seen (b) (magnification × 400).

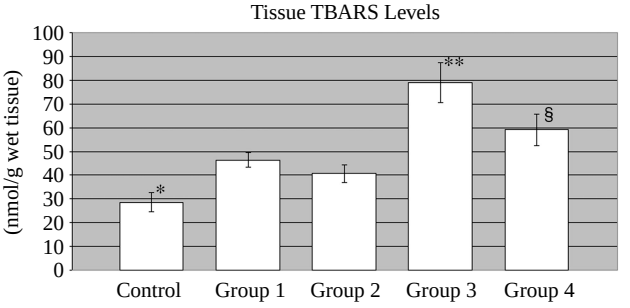

Fig. 2. Tissue thiobarbituric acid reacting substances (TBARS) levels
Group 1: 3 weeks after unilateral cavernous nerve manipulation
Group 2: 6 months after unilateral cavernous nerve manipulation
Group 3: 3 weeks after unilateral cavernous nerve resection
Group 4: 6 months after unilateral cavernous nerve resection
All values represent mean ± S.D. TBARS nmol/g wet tissue
One-way ANOVA test; F = 107.74, $P < 0.0001$
Post-hoc Tukey HSD test were used for multiple comparisons.
* $P < 0.05$ versus all
**$P < 0.05$ versus all
§$P < 0.05$ versus groups 1 and 2

SOD activity (47.43 ± 3.23) was enhanced significantly, as compared to group 1 (39.25 ± 2.54) ($P < 0.05$), but it was still lower than the control group. SOD activity was reduced in groups 1, 3 and 4 (39.25 ± 2.54, 31.42 ± 6.06 and 40.38 ± 4.24, respectively), and there were no significant differences between these three groups.

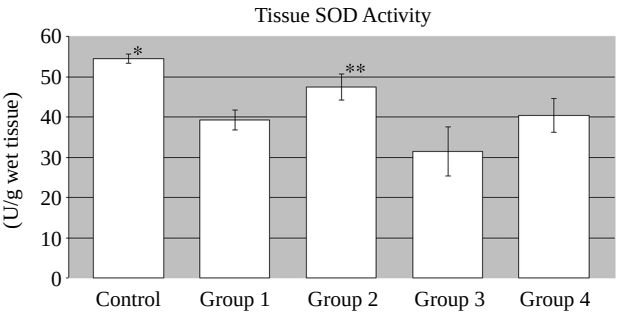

Fig. 3. Tissue superoxide dismutase (SOD) activity
Group 1: 3 weeks after unilateral cavernous nerve manipulation
Group 2: 6 months after unilateral cavernous nerve manipulation
Group 3: 3 weeks after unilateral cavernous nerve resection
Group 4: 6 months after unilateral cavernous nerve resection
All values represent mean ± S.D. SOD activity in U/g wet tissue
One-way ANOVA test; F = 47.40; $P < 0.0001$
Post-hoc Tukey HSD test were used for multiple comparisons.
*$P < 0.05$ versus all
**$P < 0.05$ versus all

Discussion

The etiologies of erectile dysfunction (ED) after nerve-sparing radical prostatectomy are still unclear. Although anatomy and neurotransmitters for innervations of the penis have been thoroughly studied, ED after unilateral and bilateral nerve-sparing pelvic surgeries is still higher than our expectations. It is highly possible that multiple pathologic mediators/ systems play a role in cavernous smooth muscle dysfunction after pelvic surgery. Evidence from basic scientific studies indicates that oxidative stress mediated through the superoxide radical (superoxide) and other reactive oxygen species (ROS) may be central to impaired cavernous function in erectile dysfunction (ED) (10). As free radicals due to oxidative stress have been accused of causing ED in diabetic rats, we can also hypothesize that oxidative stress could be one of the possible causes for ED after surgical trauma. In this study we investigated histochemical and functional changes and markers for oxidative stress (Lpx and SOD activity) after unilateral manipulation and resection of the cavernous nerve in the rat corpus cavernosum in 3 weeks and 6 months time periods.

Functional evaluations confirmed that groups 3 and 4 (unilateral nerve resection) had significantly lower intracavernous pressure (ICP) than groups 1 and 2 ($P < 0.05$) (Table 1). These results are consistent with past experience and data reported elsewhere in medical literature. Stimulation of the intact cavernous nerve revealed better intracavernous pressure in group 4 than in group 3 (58.88 ± 12.94 and 33.89 ± 16.91 cm H_2O, respectively) ($P < 0.05$). Although the intracavernous pressure of group 4 after intact cavernous nerve stimulation was higher than that of group 3 ($P < 0.05$), it was still lower than that of groups 1 and 2 (80.55 ± 9.82 and 87.22 ±9.5 cm H_2O, respectively). These results confirmed that improvement of intracavernous pressure 6 months after unilateral neurotomy was not adequate for full recovery of intracavernous pressure. Clinical experience as reported in medical literature supports these findings, i.e., that the erectile function is not recovered completely after unilateral nerve-sparing prostatectomy (6, 16).

In both neurotomy groups of our study, the NADPH diaphorase staining (NOS-positive nerve fibers) decreased extensively in the cavernous tissue comparing groups 1 and 2 (Table 2). There was an increase of NOS-positive nerve fibers of the 6 months neurotomy group, as compared with the 3 weeks neurotomy group, and it was statistically significant, (17.22 ± 6.67 and 48.89 ±19.00, respectively) ($P < 0.05$). The increase of NOS positive fibers of group 4 is also consistent with the results of the functional evaluation of the study. Autonomic nerves are known to regenerate after injury, although at varying rates (5, 7). Carrier reported that the number of NOS-containing nerve fibers within the corpus cavernosum decreased to minimum on the side of the neurotomy at 3 weeks, and by 6 months had regenerated significantly (5). In their unilateral neurotomy group, the NOS-positive nerve fibers decreased to a minimum at 3 weeks, but by 6 months had regenerated almost to the level of the intact corpus cavernorum. In carrier's study, obvious regeneration of the NOS-containing nerve fibers within the corpus cavernosum had occurred at 6 months, which was similar with our findings.

In another study, the effect of unilateral and bilateral cavernous neurotomy in the rat model has been examined (1). Basar and associates reported in the study that in the unilateral neurotomy group, NOS activity revealed as NADPH diaphorase positive staining was surprisingly increased in the sinusoids at 3 months, as compared to the bilateral neurotomy and the control group. They concluded that unilateral cavernous denervation increases NOS activity. The explanation of the authors for the possible erectile dysfunction after unilateral cavernous denervation was impairment of the pharmacological action of NO. Contrary to the last study in our experiment as mentioned above, NOS-positive fibers in corpus cavernousum is decreased at 3 weeks in the neurotomy

group, as compared to the control group and groups 1 and 2, but at 6 months after unilateral neurotomy the NADPH diaphorase positive staining increased due to regeneration of the nerve, as expected.

Oxidative stress due to surgical trauma will cause accumulation of reactive oxygen species (ROS) in the tissue, which are free radicals (14). Well-known ROS are superoxide radical (O_2^{-*}), hydroxyl radical (OH^{-*}), and hydrogen peroxide (H_2O_2). NO could also be accepted as a free radical. In pathological conditions, excessive production of NO causes this molecule to behave as reactive nitrogen radical (NO^{-*}) in the tissue (11). Low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signaling and defense against microorganisms. Nevertheless, ROS have been implicated as an important pathologic mediator in many clinical disorders. If ROS are not efficiently neutralized by an antioxidant defense system in the tissues, or if there is excessive production of ROS, which is oxidative stress, it will cause functional damage in the tissue and even cell death (15).

Evidence from basic scientific studies indicates that oxidative stress has a damaging effect on erectile function. Possible pathogenesis of oxidative stress on erectile dysfunction is increased inactivation of NO by free radicals, which results in impaired penile NO transmission and smooth muscle relaxation (10). In the condition of oxidative stress, Rajasekaran demonstrated that the radical form of NO, reactive nitrogen radical (NO^{-*}), exhibited a significant cytotoxicity to human corpus cavernosum smooth muscle cell in the culture (17). Another possible pathogenesis of oxidative stress for ED is causing angiopathy as well as functional and structural impairment of the corporal endothelium and smooth muscle. This caused ED in diabetic rats (18).

For investigation of whether a surgical trauma to the cavernous nerve, which was simulated as unilateral manipulation/dissecting from prostate tissue or resection of the cavernous nerve, could cause oxidative stress in the corpus cavernosum as compared to the control group, we used TBARS method and SOD activity as an index for oxidative stress. SOD activity, which is well known for being involved in the tissue scavenging system during oxidative stress and which protects tissue against free radicals, will be reduced with the initiation of oxidative stress in the tissues due to its excessive use by free radicals. In the experiment, the thiobarbituric acid reacting substances (TBARS) method was used as an indicator for Lpx, which is a marker of oxidative stress. TBARS test is one of the oldest and most frequently used tests for measuring the peroxidation of fatty acids on the cell membranes (8). When we compared the TBARS levels of groups 1 and 2 with the control group, there were significant increases in groups 1 and 2 (Fig. 2) ($P < 0.05$). We also found that the level of TBARS was significantly higher in groups 3 and 4, as compared to that of the other three groups. Comparison of groups 3 and 4 (nerve resection) showed that the TBARS level had been reduced in group 4 (6 months after nerve resection) ($P < 0.05$). These results demonstrated that manipulation/dissection or resection of cavernous nerve caused oxidative stress in the erectile tissue, when compared with the control group. The neurotomy procedure has caused more prominent oxidative stress than manipulation/dissection. The reason for the prominent elevation of TBARS level in the neurotomy group could be due to the majority of trauma in this group.

SOD activity of cavernous tissue, which is also another indirect indicator for oxidative stress, significantly decreased in all operated rats (manipulation or neurotomy), as compared to that of the control group (Fig. 3) ($P < 0.05$). In group 2, SOD activity increased, as compared to group 1 (47.43 ± 3.23 and 39.25 ± 2.54, respectively), but the results of the 6-month group did not reach the control group activity (47.43 ± 3.23 and 54.56 ± 1.15, respectively) ($P < 0.05$). When we compared SOD activity of groups 3 and 4 (31.42 ± 6.06 and 40.38 ± 4.25, respectively), there was an increase in group 4 (healing of oxidative stress), but it was not significant ($P < 0.05$). Impairment of SOD activity in the operated groups demonstrated that surgical procedure (unilateral manipulation or resection of nerve) has produced oxidative stress within the corpus cavernosum, which activated the scavenging system and that caused decrease of SOD activity in the cavernous tissue; in the neurotomy group, this effect is more prominent, like the TBARS levels.

The role of oxidative stress in the pathogenesis of impotence has been discussed previously. Studies on oxidative stress and ED focused mainly on the effect of ROS in diabetic animals. This study demonstrated that cavernous nerve resection or manipulation/dissection (surgical trauma) could also induce oxidative stress within the cavernous tissue. Hence, we can argue that oxidative stress may also be an etiological factor for ED after surgical pelvic trauma, as it is so in diabetic animals.

Oxidative stress after manipulation/dissection of the cavernous nerve could be an explanation of ED following bilateral nerve-sparing prostatectomy in humans. Unilateral neurotomy group animals also had exposed oxidative stress that was even more prominent than the manipulation group, and they had lower ICP during the functional evaluation than the manipulation group. In clinical practice, unilateral nerve-sparing prostatectomy patients suffer ED more often than bilateral nerve-sparing patients do.

There are few investigations in the literature to

prevent the terrible effects of oxidative stress within the corpus cavernousum. For prevention of oxidative stress, gene transfer of SOD has been investigated in aged rats (2). It was shown that gene transfer of SOD to the penis reduces superoxide radical levels and improves erectile function by inhibiting oxidative stress in aged rats. Their data provide evidence in support of the hypothesis that erectile dysfunction associated with aging is related in part to an increase in cavernosal superoxide (O_2^{-*}) formation. Gene-transfer of SOD reduces superoxide formation and restores erectile function. It may represent a novel therapeutic target for the treatment of erectile dysfunction (2). Khan *et al* demonstrated that in diabetic rabbits, reactive oxygen species might play an important role in the development of erectile dysfunction due to impaired smooth muscle relaxation, where SOD as well significantly reversed the impaired relaxation (13). These therapeutic approaches, that is, therapy against oxidative stress, may also find clinical applications to restore erectile function in ED after surgical trauma. Hence, further investigation is needed.

In conclusion, this study confirmed that unilateral cavernous neurotomy caused a decrease of intra cavernous pressure and NOS fibers. Oxidative stress, which occurred after either unilateral manipulation/dissection or resection of cavernous nerve, was more prominent in the nerve resection group. Based on the data presented here, we could conclude that neurotomy and surgical manipulation of the cavernous nerve caused oxidative stress in rat corpus cavernosum. This data provided evidence in support of the hypothesis that erectile dysfunction associated with cavernous nerve manipulation or resection was related in part to oxidative stress in the corpus cavernosum. Although antioxidant treatment improved erectile function in aged or diabetic rats, further investigation is needed to find out whether antioxidant treatment could also prevent erectile dysfunction after surgical trauma.

Acknowledgment

We are grateful to M.Sc. Ömer Uysal (Istanbul University, Cerrahpasa Medical Faculty, Department of Biostatistics) for assistance in statistical analyses of the study.

References

1. Basar, M.M., Yıldız, M., Söylemezoğlu, F., Surucu, H.S., Başar, H., Akan, H. and Başar R. Histopathological changes and nitric oxide synthase activity in corpous cavernosum from rats with neurogenic erectile dysfunction. *Br. J. Urol. Int.* 83: 101-107, 1999.
2. Bivalacqua, T.J., Armstrong, J.S., Biggerstaff, J., Abdel-Mageed, A.B., Kadowitz, P.J., Hellstrom, W.J.G. and Champion, H.C. Gene transfer of extracelluler SOD to penis reduces O and improves erectile function in aged rats. *Am. J. Physiol.* 284: 1408-1421, 2003.
3. Buege, J.A. and Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol*. 12: 302-310, 1978.
4. Burnett, A.L., Lowenstein, C.J., Bredt, D.S., Chang, T.S.K. and Synder, S.H. Nitric oxide: a physiologic mediator of penile erection. *Science* 257: 401-404, 1992.
5. Carrier, S., Zvara, P., Nunes, L., Kour, N.W., Rehman, J. and Lue, T.F. Regeneration of nitric oxide synthase-containing nerves after cavernous nerve neurotomy in the rat: *J. Urol*. 153: 1722-1727, 1995.
6. Catalona, W.J. and Basler, J.W. Return of erection and urinary continence following nerve sparing radical retro pubic prostatectomy. *J. Urol*. 150: 905- 909, 1993.
7. Gibson, G.R. Impotence following fractured pelvis and ruptured urethra. *Br. J. Urol*. 42: 86-89, 1970.
8. Gutteridge, J.M.C. and Quilan, G.J. MDA formation from lipid peroxides in the TBA test: the role of lipid radicals, iron salts, and metal chelators. *J. Appl. Biochem*. 5: 293-299, 1983.
9. Hope, B.T., Michael, G.J., Knigne, K.M. and Vincent, S.R. Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc. Natl. Acad. Sci. USA.* 88: 2811-2814, 1991.
10. Jones, R.W., Rees, R.W., Minhas, S., Ralph, D., Persad, R.A. and Jeremy, J.Y. Oxygen free radicals and the penis. *Expert Opin. Pharmacother*. 3: 889-897, 2002.
11. Jung, G.W., Spencer, E.M. and Lue, T.F. Growth hormone enhances regeneration of nitric oxide synthase-containing penile nerves after cavernous nerve neurotomy in rats. *J. Urol*. 160: 1899-1904. 1998.
12. Kelm M. Nitric oxide metabolism and breakdown. *Biochim. Biophys. Act*a. 1411: 273-289, 1999.
13. Khan, M.A., Thompson, C.S., Jeremy, J.Y., Mumtaz, F.H., Mikhailidis, P. and Morgan, R.J. The effect of superoxide dismutase on nitric oxide-mediated and electrical field-stimulated diabetic rabbit cavernosal smooth muscle relaxation. *BJU Int*. 87: 98-103, 2001.
14. Kreinhoff, U., Elmadfa, I., Salomon, F. and Weidler, B. Antioxidant status after surgical stress. *Infusionstherapie* 17: 261-267, 1990.
15. Mates, J.M., Perez-Gomez, C. and Nunez de Castro, I. Antioxidant enzymes and human diseases. *Clin. Biochem*. 32: 595-603, 1999.
16. Mirone, V., Imbimbo, C., Palmieri, A., Longo, N. and Fusco, F. Erectile dysfunction after surgical treatment. *Int. J. Androl.* 26: 137-140, 2003.
17. Rajasekaran, M., Hellstrom, W.J. and Sikka, S.C. Nitric oxide induces oxidative stress and mediates cytotoxicity to human cavernosal cells in culture. *J. Androl*. 22: 34-39 , 2001.
18. Ryu, J.K., Kim, D.J., Lee, T., Kang, Y.S.,Yoon, S.M. and Suh, J.K. The role of free radical in the pathogenesis of impotence in streptozotocin induced diabetic rats. *Yonsei Med. J*. 44: 236-241, 2003.
19. Sun, Y., Oberley, L.W. and Li, Y.A. A simple method for clinical assay of super oxide dismutase. *Clin. Chem.* 34: 497-500, 1998.
20. Walsh, P.C., Epstein, J.I. and Lowe, F.C. Potency following radical prostatectomy with wide unilateral excision of the neurovascular bundle. *J. Urol*. 138: 823-828, 1987.