ORIGINAL RESEARCH—BASIC SCIENCE

Nitrergic Function Is Lost but Endothelial Function Is Preserved in the Corpus Cavernosum and Penile Resistance Arteries of Men after Radical Prostatectomy

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ABSTRACT

Introduction. Radical prostatectomy (RP) frequently results in erectile dysfunction (ED). It has been hypothesized that alterations of cavernosal tissue subsequent to RP contribute to ED but functional evaluation of the impact of RP on human erectile structures is lacking.

Aim. This study aims to evaluate endothelial function of human corpus cavernosum (HCC) and human penile resistance arteries (HPRA) and neurogenic responses of HCC from patients with ED secondary to RP (ED-RP).

Methods. HCC strips and HPRA were obtained from organ donors without history of ED (No-ED) and patients with ED who were segregated depending on ED etiology: ED-RP or vasculogenic (ED-VASC). Functional evaluation of HCC and HPRA was performed in organ chambers and wire myographs, respectively. Histological evaluation of cavernosal tissue consisted of trichrome staining for fibrosis quantification and TUNEL assay for determination of apoptosis.

Main Outcome Measures. Endothelium-dependent and endothelium-independent relaxation, electrical field stimulation (EFS)-induced neurogenic contraction and relaxation, and cavernosal fibrosis and apoptosis.

Results. Endothelium-dependent relaxations were significantly impaired in HCC and HPRA from ED-VASC patients while these responses in ED-PR patients were not different to No-ED. Similarly, sildenafil-induced relaxations were reduced in HCC and HPRA from ED-VASC but were preserved in ED-RP. Adrenergic contractions induced by EFS in HCC were potentiated in both ED-RP and ED-VASC. EFS-induced nitrergic relaxation was significantly reduced in HCC from ED-VASC but was almost abolished in ED-RP. Fibrous tissue content and cavernosal apoptosis in HCC from ED-RP were not significantly different from No-ED.

Conclusions. Endothelial function and cavernosal sensitivity to phosphodiesterase type 5 inhibitors are preserved in erectile tissue from ED-RP while a marked imbalance in neurogenic modulation of cavernosal tone favoring adrenergic contractile responses over nitrergic relaxation is manifested. Fibrotic and apoptotic processes in cavernosal tissue are not specifically associated to ED-RP. These evidences could help to retarget therapeutic strategies in the management of ED after RP. **Martínez-Salamanca JI, La Fuente JM, Fernández A, Martínez-Salamanca E, Pepe-Cardoso AJ, Carballido J, and Angulo J. Nitrergic function is lost but endothelial function is preserved in the corpus cavernosum and penile resistance arteries of men after radical prostatectomy. J Sex Med 2015;12:590–599.**

Key Words. Erectile Dysfunction; Radical Prostatectomy; Human Corpus Cavernosum; Endothelium-Dependent Relaxation; Nitrergic Relaxation; Penile Rehabilitation

Introduction

Radical prostatectomy (RP) remains the reference treatment for organ-confined prostate cancer in patients with a life expectancy of 10 years or more [1], but despite the advent of nerve sparing techniques, it often causes sustained erectile dysfunction (ED) [2,3]. Postprostatectomy erectile dysfunction (ED-RP) typically results from injury to the cavernous nerves that course along the posterolateral aspects of the prostate and provide most of the autonomic input to the erectile tissue. It is estimated that 10% of all men experiencing ED suffer the condition as a result of surgical procedure, typically, radical pelvic surgery. Major discrepancy exists in the literature regarding postoperative potency rates with spontaneous erectile function occurring in 20–80% of patients [4–8].

Patients with ED-RP are poor responders to phosphodiesterase type 5 inhibitors (PDE5) [9], which represent the first-line therapy for the treatment of ED. Nevertheless, early and continuous administration of these compounds have been proposed to prevent the progression of fibrosis [10,11] and produce a positive effect on erectile function in ED-RP, but the recovery of erectile function is obtained in a limited percentage of patients [12–15].

It is thought that RP causes structural and functional alterations in erectile tissue through inducing hypoxia/fibrosis, vascular insufficiency, and/or neurological degeneration. However, this is based on results obtained in rat models of cavernous nerve injury [16–18] and a very limited number of histological/immunohistological studies in human cavernosal tissue [19,20]. Thus, functional evaluation of human erectile tissue after RP in comparison with other types of ED is definitely lacking.

The aim of this work was to evaluate the impact of RP on endothelial and neurogenic responses of human corpus cavernosum (HCC) and human penile resistance arteries. The functional characteristics of erectile tissue from patients with ED-RP were compared with those from patients with vascular ED, and structural alterations of cavernosal tissue were also evaluated.

Materials and Methods

Human Tissues

HCC specimens were obtained from organ donors without history of ED (No-ED) at the time of organ collection for transplantation and from men with ED who gave written informed consent at the time of penile prosthesis implantation. ED patients were segregated depending on ED etiology: secondary to RP (ED-RP) or due to vascular causes (ED-VASC). Organ donors relatives signed consent for tissue collection for research purposes following Portuguese rules and the protocol approved by the Hospital Santo Antonio, Porto, Portugal. Protocols and consent forms signed by ED patients were approved by the Ethic Committees at the hospitals where the tissues were collected in Portugal and Spain (081/10(059-DEFI/077-CES) and Acta 256/28-06-10). Tissues were maintained at 4–6°C in M-400 solution (composition per 100 mL: mannitol, 4.19 g; KH_2PO_4, 0.205 g; $K_2HPO_4{\cdot}3H_2O$, 0.97 g; KCl, 0.112 g; $NaHCO_2$, 0.084 g; pH 7.4) until their use, which ranged between 16 and 24 hours from extraction [21,22].

Functional Evaluation of Corpus Cavernosum

Strips of corpus cavernosum tissue ($3 \times 3 \times 7$ mm) were immersed in 8 mL organ chambers containing Krebs–Henseleit solution (KHS) of the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11, KH_2PO_2 1.2, ethylenediamine tetraacetic acid (EDTA) 0.027, maintained at 37°C and aerated with 95% O_2/5% CO_2, pH 7.4, for isometric tension recording as previously described [21,22]. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 μM phenylephrine (PE). The preparations were then exposed to 120 mM K^+, and the contractile response was measured. Relaxation responses were evaluated in strips contracted with PE (1–3 μM; 80% of K^+-induced contraction) by cumulative additions of compounds to the chambers. Electrical field stimulation (EFS) was applied to HCC strips by means of two platinum electrodes placed at both sides of the tissue and connected to a current stimulator (Cibertec, Madrid, Spain). Parameters of EFS were: 75 mA, 0.5 ms for 20 seconds. Neurogenic contractile responses to EFS (0.5–48 Hz) were obtained in untreated, noncontracted strips. For evaluating nitrergic relaxations, the tissues were pretreated with atropine (0.1 μM) and guanethidine (30 μM), and EFS was applied at 0.5–16 Hz to HCC strips precontracted with PE (1–3 μM).

Vascular Reactivity of Human Penile Resistance Arteries

Penile small arteries, helicine arteries (lumen diameter 150–400 μm), were dissected by carefully

removing the adhering trabecular tissue, and arterial ring segments (2 mm long) were subsequently mounted on wire myographs (J.P. Trading, Aarhus, Denmark) for isometric tension recordings as previously described [21,22]. The vessels were allowed to equilibrate for 30 minutes in KHS at 37°C continuously bubbled with 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. The arteries were then set to 90% of determined internal circumference under a transmural pressure of 100 mm Hg (L_{100}), at which the force development was close to maximal. The preparations were then exposed to 120 mM K^+, and the contractile response was measured. The arteries were contracted with 1–3 μM norepinephrine (80% of K^+-induced contraction approximately) and relaxation responses were evaluated by cumulative additions of compounds to the chambers.

Determination of Cavernosal Apoptosis and Fibrosis

Apoptosis was determined by terminal 2 -deoxyuridine-5 -triphosphate nick-end-labeling (TUNEL) assay in deparaffined tissue sections (6 μm) of corpus cavernosum. A uorescence-based commercial kit was used following manufacturer s specifications (Promega Biotech Ibérica, Alcobendas, Spain). Percentage of apoptosis was calculated by counting apoptotic cell nuclei (TUNEL-positive cells) relative to total number of cell nuclei in six high-magnification visual fields (×400) for each sample. For determination of cavernosal fibrosis, deparaffined tissue sections (6 μm) were stained with Masson s trichrome and the percentage of the area stained in blue (fibrotic tissue) with respect to total area of high-magnification visual fields (×200) (five per patient) was calculated by using morphometric software (Image J, National Institutes of Health, Bethesda, MD, USA). Photograph capture and quantification were performed by two different investigators who were unaware of the classification of the analyzed tissue.

Data Analysis

Relaxation responses are expressed as the percentage of total relaxation (loss in tone) induced by the addition of 0.1 mM papaverine to the chambers at the end of the experiment. pD_2 is defined as the –log molar concentration required to obtain 50% of maximal relaxation. It was graphically calculated for each individual curve. EFS-induced contractions are expressed as the percentage of the contraction elicited by 120 mM K^+ in each tissue. All data are expressed as mean ± standard error. Complete concentration-response or frequency-response curves were obtained and compared by a two-factor analysis of variance (ANOVA) statistical test using StatView software for Apple computers (SAS, Cary, NC, USA) [23]. All other data were compared by one-factor ANOVA, followed by a Student–Newmann–Keuls posttest (GraphPad InStat, San Diego, CA, USA).

Results

Endothelium-Dependent Relaxation Is Preserved in Both HCC and Penile Resistance Arteries (HPRA) from ED-RP

Main characteristics of patients from whom the tissues were collected are displayed in Table 1. As expected, an elevated presence of cardiovascular risk factors and cardiovascular disease was found in ED-VASC while only the frequency of hypertension was significantly elevated in ED-RP with respect to No-ED subjects.

Table 1 Main characteristics of the patients

	No ED	ED-VASC	ED-RP
n	20	30	29
Age, years	55.0 ± 2.9	58.3 ± 1.2	60.9 ± 1.5
(range)	(23–76)	(41–70)	(44–72)
Diabetes (%)	0 (0)	15 (50.0)***	1 (3.4)†††
Hypertension (%)	2 (10)	17 (56.7)***	10 (34.5)*
Dyslipidemia (%)	0 (0)	9 (30.0)**	5 (17.2)
Obesity (%)	0 (0)	3 (10.0)	0 (0)
Cardiovascular disease (%)	0 (0)	5 (16.7)	0 (0)
IIEF-5 score (range)	—	7.8 ± 1.5 (5–9)	8.0 ± 1.5 (5–10)
Time from prostatectomy, months	—	—	34.6 ± 5.0

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. No-ED and †††$P < 0.001$ vs. ED-VASC by Fisher's exact test.
No-ED = organ donors without history of erectile dysfunction (ED); ED-VASC = patients with ED of vascular aetiology; ED-RP = patients with ED after radical prostatectomy; IIEF-5 = International Index of Erectile Function—erectile function domain.

Table 2 Parameters of endothelium-dependent relaxation induced by acetylcholine in human corpus cavernosum (HCC) and penile resistance arteries (HPRA) from different patient groups and subgroups

		HCC		HPRA	
		pD_2	E_{max} (%)	pD_2	E_{max} (%)
No-ED		6.39 ± 0.24 n = 6	76.3 ± 5.9	7.31 ± 0.29 n = 6	88.2 ± 3.5
ED-VASC		5.41 ± 0.21** n = 10	53.4 ± 5.1*	5.57 ± 0.30*** n = 9	57.6 ± 5.7***
ED-RP		6.49 ± 0.13†† n = 10	73.5 ± 2.5†	7.13 ± 0.18††† n = 11	88.0 ± 2.0†††
ED-RP subgroups					
	Rehab	6.47 ± 0.03 n = 4	76.2 ± 2.9	6.96 ± 0.52 n = 4	89.9 ± 3.3
	No Rehab	6.50 ± 0.16 n = 6	71.1 ± 3.2	7.18 ± 0.17 n = 7	87.1 ± 3.0
	NSRP	6.42 ± 0.16 n = 4	75.2 ± 2.9	6.99 ± 0.33 n = 4	90.8 ± 3.1
	Non-NSRP	6.54 ± 0.20 n = 6	72.1 ± 3.4	7.19 ± 0.22 n = 7	87.6 ± 2.6

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. No-ED, and †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ vs. ED-VASC by one-factor ANOVA followed by Student–Newmann–Keuls test. No significant differences between ED-RP subgroups were obtained.

pD_2 is defined as the –log M of the concentration of acetylcholine (ACh) to obtain 50% of total relaxation. E_{max} is the maximum percentage of relaxation achieved with any ACh concentration. Data are expressed as mean ± SEM. NSRP indicates nerve-sparing radical prostatectomy. Rehab indicates penile rehabilitation with PDE5 inhibitors. n indicates the number of patients included for determinations.

No-ED = organ donors without history of erectile dysfunction (ED); ED-VASC = patients with ED of vascular aetiology; ED-RP = patients with ED after radical prostatectomy.

Endothelium-dependent relaxations induced by acetylcholine (ACh, 1 nM to 10 μM) in HCC were significantly reduced in ED-VASC when compared with those obtained in HCC from No-ED. In contrast, endothelium-dependent relaxations in HCC from ED-RP were not impaired (Table 2) (Figure 1A). Similar results were obtained when endothelium-dependent vasodilation was evaluated in HPRA, as ACh-induced vasodilations were significantly impaired in HPRA from ED-VASC with respect to No-ED but those in ED-RP remained unaltered (Table 2) (Figure 1B).

Preservation of endothelial function was not due to the application of penile rehabilitation procedures as a small number of patients from our sample underwent this clinical approach after RP. In fact, responses to ACh in penile tissues from this specific population were not different from that obtained from ED-RP patients who did not

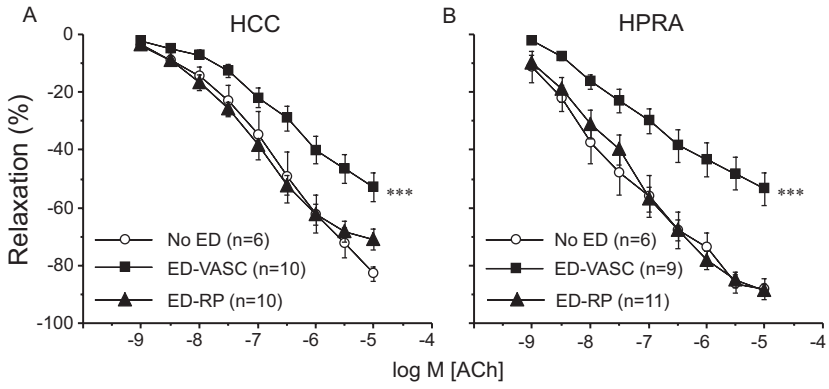

Figure 1 Endothelium-dependent relaxations induced by acetylcholine (ACh; 1 nM to 10 μM) in human corpus cavernosum strips (HCC) (A) and human penile resistance arteries (HPRA) (B) from organ donors without history of ED (No ED), patients with ED not related to radical prostatectomy (ED-VASC) or patients with ED secondary to radical prostatectomy (ED-RP) previously contracted with phenylephrine (1–3 μM) (1.87 ± 0.49, 1.70 ± 0.23 and 1.69 ± 0.19 g, respectively; $P = 0.9102$) and norepinephrine (1–3 μM) (7.55 ± 0.50, 7.28 ± 0.98 and 7.35 ± 0.82 mN, respectively; $P = 0.7613$), respectively. Data are expressed as mean ± SEM of the percentage of maximal relaxation induced by papaverine (0.1 mM). n indicates the number of patients. *** indicates $P < 0.001$ vs. No-ED or ED-RP by a two-factors ANOVA.

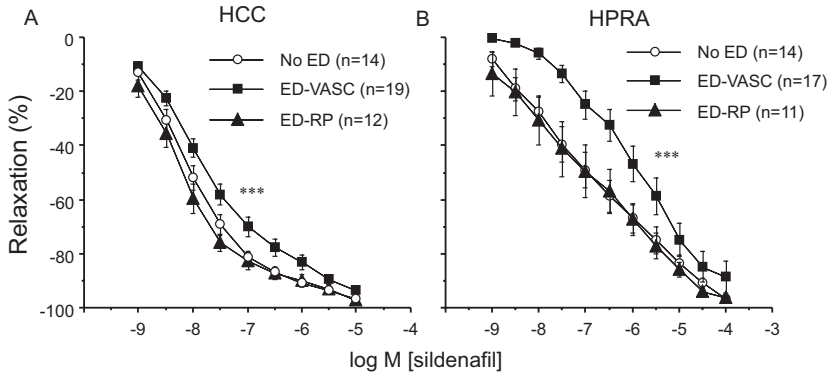

Figure 2 Relaxations induced by the PDE5 inhibitor, sildenafil (1 nM to 10 μM) in human corpus cavernosum strips (HCC) (A) and human penile resistance arteries (HPRA) (B) from organ donors without history of ED (No-ED), patients with ED not related to radical prostatectomy (ED-VASC) or patients with ED secondary to radical prostatectomy (ED-RP) previously contracted with phenylephrine (1–3 μM) (1.99 ± 0.36, 1.84 ± 0.27 and 1.95 ± 0.32 g, respectively; $P = 0.9363$) and norepinephrine (1–3 μM) (6.73 ± 0.68, 7.65 ± 1.22 and 7.58 ± 0.87 mN, respectively; $P = 0.5991$), respectively. Data are expressed as mean ± SEM of the percentage of maximal relaxation induced by papaverine (0.1 mM). n indicates the number of patients. *** indicates $P < 0.001$ vs. No-ED or ED-RP by a two-factors ANOVA.

undergo penile rehabilitation with PDE5 inhibitors (Table 2). In the same way, adoption of nerve sparing techniques for RP did not modify the endothelium-dependent relaxations (Table 2).

Similarly, relaxations to the PDE5 inhibitor, sildenafil, were significantly impaired in both HCC and HPRA from ED-VASC, whereas the relaxant and vasodilatory capacities of the PDE5 inhibitor were preserved in HCC and HPRA from ED-RP (Figure 2). HCC from ED patients, either from ED-VASC or ED-RP, displayed unaltered endothelium-independent relaxations to the NO-donor, sodium nitroprusside (SNP; 1 nM to 10 μM) when compared with HCC from No-ED (pD_2 7.39 ± 0.07, 7.31 ± 0.06 and 7.34 ± 0.09 for No-ED, ED-VASC, and ED-RP, respectively; n.s.).

ED Secondary to RP Is Associated to an Impairment of Nitrergic Relaxation and Enhanced Neurogenic Contraction of HCC

In HCC strips contracted with PE and treated with guanethidine (30 μM) to inhibit adrenergic neurotransmission, and atropine (0.1 μM) to block muscarinic receptors, EFS application (0.5–16 Hz) resulted in frequency-dependent relaxations. Relaxations were nitrergic in nature since were abolished by inhibiting NO synthesis with L-NAME (100 μM) (Figure 3A). Nitrergic relaxations were significantly reduced in HCC from ED-VASC but were more profoundly impaired in HCC from ED-RP (Figure 3C and D). In contrast, neurogenic contractions of HCC in response to EFS (0.5–48 Hz) were not simply preserved but were significantly enhanced in ED-RP to the same extent as those in ED-VASC (Figure 3E). Contractile responses induced by EFS were mediated by adrenergic system as they were prevented by α-adrenergic receptor blockade with phentolamine (1 μM) (Figure 3B). The potentiated response was not related to an alteration of contractile responses to adrenergic receptor stimulation of the ED-RP tissues as contractions induced by PE for relaxation experiments were not significantly different among the three groups of patients (2.05 ± 0.24, 1.84 ± 0.17, and 1.85 ± 0.16 g for No ED, ED-VASC, and ED-RP, respectively; n.s.). ED-VASC and ED-RP were not only associated to enhanced contractility of HCC to EFS but were also related to increased sensitivity to neurogenic stimulation because the frequency required for obtaining 50% of maximal response (EF_{50}) in HCC from these patients was significantly lower (27.0 ± 0.9, 18.5 ± 2.0 and 19.9 ± 0.9 Hz for No ED, ED-VASC and ED-RP, respectively; $P < 0.001$ for both groups of ED patients vs. No-ED).

Nitrergic relaxations in HCC from ED-RP patients who underwent nerve-sparing surgery for RP (NSRP) were not significantly different from those ED-RP not specifically submitted to NSRP (non-NSRP). Maximal relaxation to EFS was 16.1 ± 5.5% for non-NSRP (n = 9) and 24.5 ± 6.1% for NSRP (n = 5; n.s.). Similarly, the enhancement of neurogenic contractions was also present in ED-RP patients undergoing NSRP. In fact, EFS-induced contractions tended to increase in this specific population (E_{max} 15.9 ± 4.8% vs.

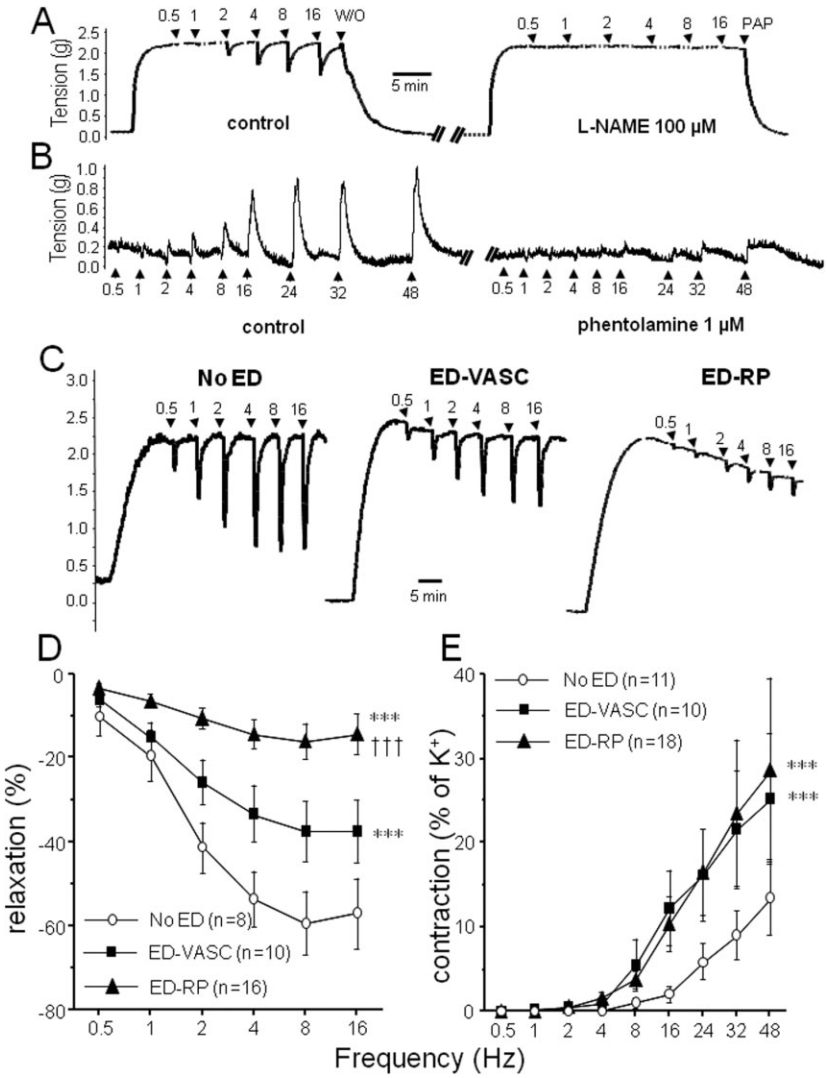

Figure 3 Panel A shows a representative tracing of neurogenic relaxation induced by electrical field stimulation (EFS) in a human corpus cavernosum (HCC) strip contracted with phenylephrine (1 μM) and treated with guanethidine (30 μM) and atropine (0.1 μM) that was prevented by inhibition of NO synthesis with N^G-nitro-L-arginine-methyl-ester (L-NAME, 100 μM). Panel B shows a representative tracing of neurogenic contraction induced by EFS in an untreated HCC strip that was prevented by α-adrenergic receptor blockade with phentolamine (1 μM). HCC strips in panels A and B were obtained from a patient with ED not related to radical prostatectomy (ED-VASC) although this result is applicable to any other patient group. Panel C shows representative nitrergic relaxations in HCC strips contracted with PE (1 μM) from an organ donor without history of ED (No-ED), an ED-VASC patient and a patient with ED secondary to radical prostatectomy (ED-RP). Lower panels show quantification of nitrergic relaxations in HCC strips contracted with PE (1–3 μM) (2.33 ± 0.43, 2.16 ± 0.47 and 1.90 ± 0.31 g, respectively; $P = 0.7399$) (D) and adrenergic contractions in untreated HCC strips (E). Data are expressed as mean ± SEM of the percentage of maximal relaxation induced by papaverine (0.1 mM) (D) and as mean ± SEM of the percentage of contraction elicited by 120 mM K^+ (E). n indicates the number of patients. *** indicates $P < 0.001$ vs. No-ED, ††† $P < 0.001$ vs. ED-VASC by a two-factor ANOVA.

47.1 ± 24.6% for non-NSRP [n = 11] and NSRP [n = 5], respectively; n.s.).

The time passed from RP did not seem to be critical for the manifestation of altered neurogenic imbalance in HCC from ED-RP. This is based on the lack of significant correlation between time after RP and maximal nitrergic relaxation ($r^2 = 0.001$; n.s.) or maximal adrenergic contraction ($r^2 = 0.093$; n.s.).

Cavernosal Apoptosis and Fibrosis Are not Specifically Linked to ED Secondary to RP

Fibrotic tissue content in HCC was slightly but significantly elevated in ED-VASC. This signifi-

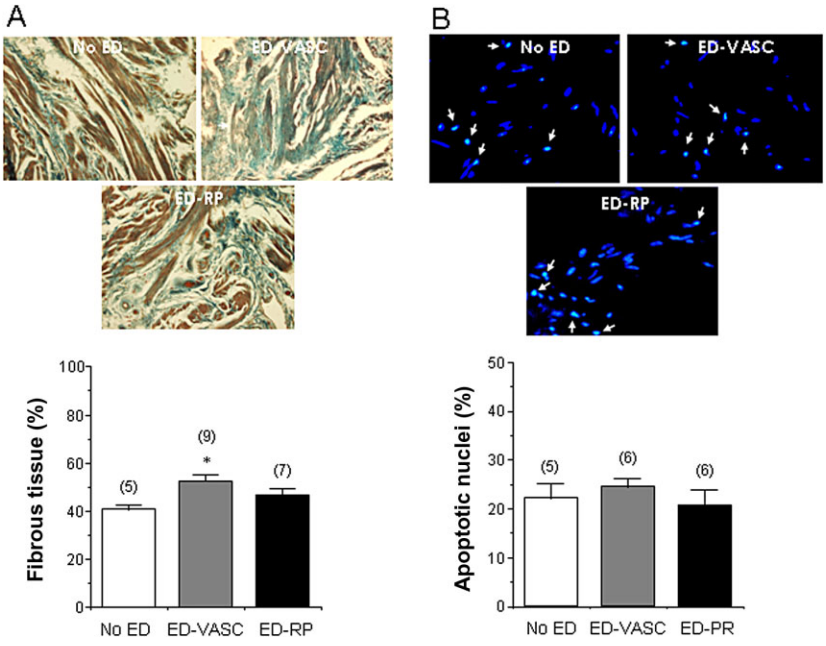

Figure 4 Detection of fibrosis by trichrome staining (A) and apoptosis by TUNEL (B) in human corpus cavernosum (HCC) from organ donors without history of ED (No-ED), patients with ED not related to radical prostatectomy (ED-VASC) or patients with ED secondary to radical prostatectomy (ED-RP). Data are expressed as mean ± SEM of the percentage of the area occupied by fibrotic tissue in each field (×200) (A) and as mean ± SEM of the percentage of apoptotic nuclei (B). n indicates the number of patients. * indicates $P < 0.05$ vs. No ED by a one-factor ANOVA followed by Student-Newmann-Keuls test. Upper panels show representative images. Arrows indicate apoptotic nuclei.

cant increase in fibrosis was not manifested in HCC from ED-RP, although fibrous tissue content in HCC from these patients was not significantly different from that determined in ED-VASC (Figure 4A). There were no significant differences among the groups of subjects with respect to the presence of apoptosis in cavernosal tissue (Figure 4B).

Discussion

It has been proposed that neurapraxia/damage of cavernous nerves during radical pelvis surgery causes erectile incapacity, cavernosal in ammation, and hypoxia leading to defective nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway and oxidative stress that harm endothelium and smooth muscle resulting in cavernosal fibrosis. Then, when this process is completed, a potential nerve restoration could find an unresponsive erectile tissue that would impede erectile function recovery and would explain the high rate of permanent ED after RP even when nerve sparing techniques are applied [9,24]. This view of ED-RP pathophysiology represents the rationale for penile rehabilitation that has been extensively evaluated and widely applied to patients undergoing RP, mainly by chronic administration of PDE5 inhibitors. However, although based on very reasonable concepts, the research evidence supporting this hypothesis is limited to animal models that manifest substantial morphological alterations of penile tissue architecture, including increased apoptosis, smooth muscle/endothelium loss and fibrosis [16–18,25,26], and to scarce histological evaluation of human tissue after RP [19,20].

Reduced number of elastic fibers, decreased smooth muscle, and increased collagen content has been found in cavernosal biopsies from men undergoing RP [19]. However, these observations were not compared with other types of ED. In fact, in a general sample, the reduction in elastic fibers, smooth muscle, and endothelium content was more notable in ED patients with arterial disease, elevated age, diabetes and smoking habit [27]. A recent study showed significantly increased fibrosis and apoptosis in corpus cavernosum from prostatectomized patients with severe ED but these alterations were not significantly different from those in diabetic patients and even were lower in magnitude [20]. In addition, the status of prostatectomy population with respect to the presence of vascular risk factors was not reported.

To our knowledge, we provide the first functional evaluation of human erectile tissue obtained from patients with ED secondary to RP in comparison with healthy specimens and with those from patients with vasculogenic ED. Our results do not support a generalized degeneration of cavernosal tissue after RP as endothelial and smooth muscle relaxations are preserved in penile tissues. Accordingly, the relaxant capacity of the PDE5 inhibitor, sildenafil, is conserved intact in HCC and HPRA from ED-RP suggesting that the failure to PDE5

inhibition therapy does not relate to defective activity or tissue inability to relax. In fact, none of the non-neurogenic relaxant responses mediated by NO/cGMP pathway, namely ACh, sildenafil, and SNP, is altered in erectile tissues from ED-RP. In contrast, ED-VASC is associated with impaired endothelial relaxation and significantly reduced efficacy of sildenafil to relax HCC and to vasodilate HPRA, as previously reported [21].

The outstanding feature that specifically characterizes functional alterations induced by RP in human cavernosal tissue is a profound impairment of nitrergic relaxation. HCC from ED-RP displays nitrergic relaxations that are not only reduced with respect to healthy tissue but also are decreased with respect to ED-VASC, who manifest a milder reduction. Severe impairment of nitrergic responses is not related to a generalized deficit in neurogenic responses as adrenergic contractions induced by EFS in HCC from ED-RP are not reduced. Furthermore, these contractions are enhanced with respect to No-ED to the same extent as that observed in HCC from ED-VASC. The loss of nitrergic function could be responsible for this enhancement of neurogenic contractions in cavernosal tissues from ED-RP as NO/cGMP pathway modulates EFS-induced noradrenergic contractions in HCC [28]. Thus, neurogenic control of cavernosal tone in ED-RP is imbalanced favoring contractile responses over relaxant input. Studies using rat models of cavernous nerve injury (CNI) have consistently reported nitrergic degeneration in cavernosal tissue determined as a loss in nNOS positive nerve fibers [29,30]. In contrast, functional evaluation of rat corpus cavernosum after CNI demonstrated blunted nitrergic relaxations despite good regeneration of nitrergic nerves [31]. Although altered NO-mediated cavernosal smooth muscle relaxation was proposed for explaining this observation, it could be related to aberrant synapse formation in neuroregeneration after CNI [32]. In fact, in our human tissue model, none of the non-neurogenic relaxant responses mediated by NO/cGMP pathway are altered in erectile tissues from ED-RP.

On the other hand, the here reported enhancement of neurogenic contractions has no clear antecedent as the in uence of CNI on sympathetic nerves has not been previously addressed. However, the specific contribution of this alteration to ED after CNI deserves evaluation in the future since erectile function under these conditions could potentially benefit from adrenergic modulation.

Morphological evaluation of cavernosal specimens yielded results consistent with functional preservation of endothelial and smooth muscle responses, as fibrosis is not significantly increased in HCC from ED-RP, whereas tissues from ED-VASC display a significant elevation of fibrotic content. Furthermore, no evidence of increased apoptosis is detected in human cavernosal tissue from either ED-RP or ED-VASC. We did not find the significant fibrotic alterations previously described in cavernosal tissue from ED patients undergoing RP [19,20]. Although this could be due to differences in study population (no information on the presence or the absence of vascular risk factors was provided in those studies), our observations are supported by functional evidence. Furthermore, this concept would be consistent with the high efficacy of intracavernosal injections in postprostatectomy ED patients [33]. Based on our results, morphological aspect of human cavernosal tissue after RP substantially differs from that in rat cavernosal tissue in models of CNI, suggesting that differences between human disease and animal models could exist.

The present results do not support the widely accepted concept that rehabilitation with PDE5 inhibitors should be required for preservation of cavernosal endothelium and smooth muscle and would rather support critical points of view [34] as endothelial and smooth muscle relaxant capacity are preserved in ED-RP even in the absence of penile rehabilitation. This could explain the limited clinical improvement of erectile function obtained with PDE5 inhibitor administration right after surgery [13–15] despite the demonstrated clear beneficial impact on erectile function and cavernosal morphology in animal models of CNI when PDE5 inhibitors were continuously administered [29,30,35]. However, a potential benefit, related or not to endothelial function, driven by continuous PDE5 inhibitor administration cannot be discarded as some studies have yielded positive results with this approach [12]. In this sense, some preclinical studies suggest that PDE5 inhibition may increase blood ow to ganglia of nitrergic neurons preserving nitrergic function and facilitating nitrergic regeneration [36,37], although the positive effects of PDE5 inhibitors could be potentially contributed by the ability of these drugs to promote neurogenesis [38,39].

Despite the value of experimentation with human tissues, these studies bear some unavoidable limitations. In this sense, we lack information on the function of cavernosal tissue from patients

undergoing RP but recovering erectile function. Collection of cavernosal tissue valid for functional assays from these patients is not possible as they do not require prosthesis implantation. In the same sense, it is not possible to afford longitudinal studies to explore the time-frame of functional alterations. On the other hand, the morphological analysis of nitrergic fibers in tissues from the different groups of subjects including those who were able to recover erectile function deserves future research efforts to complete the present functional findings.

Conclusions

An imbalance of neurogenic control of cavernosal tone favoring contractile responses over nitrergic relaxations is the main functional alteration of the erectile tissue from ED-RP, while endothelium- and smooth muscle-mediated relaxations are preserved. These results obtained after the first functional evaluation of human ED-RP tissues in comparison with both No ED and ED-VASC suggest that strategies directed to reverse neurogenic imbalance by recovering/preserving nitrergic responses and avoiding adrenergic enhancement could be of relevance in the management of ED after RP rather than those targeted to preserve endothelial and smooth muscle function.

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