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ORIGINAL ARTICLE

The effects of different vasovasostomy techniques on motility of vas deferens (vas motility following vasovasostomy)

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Abstract

Purpose Vasovasostomy is used to correct vas deferens (VD) transections encountered during surgery or to reverse sterilization vasectomies. Achieving vasal patency is the primary goal and the success is assessed on various factors including VD patency, flow rates, and pregnancy rates. While preserving vas motility is not a major concern in surgical practice, it is worth noting that VD has peristaltic activity which plays crucial role during ejaculation. Any disruption in its motility could potentially lead to negative outcomes in the future. We conducted an experimental study to assess vas motility changes following vasovasostomy.

Methods The study was approved by Gazi University, Animals Ethic Committee. Twenty-four rats were allocated to four groups. Left-sided VD was harvested in control group (Gr1). The rest of the animals were subjected to transection of VD. Gr2 and 3 underwent microscopic and macroscopic anastomosis, respectively, while Gr4 underwent vasal approximation. After 12 weeks, all left-sided VD were resected, electrical field stimulation (EFS) and exogenous drugs were applied to induce contractions. Statistical analyses were performed and p value < 0.05 was regarded as statistically significant.

Results The first and second phases of EFS-induced contractile responses(CR) increased for Gr3 and decreased for Gr4 at submaximal and maximal frequencies. An increase only at maximal frequency for second phase EFS-induced CR was encountered for Gr2. α-β-methylene-ATP-induced CR decreased for Gr3 and 4. Noradrenaline-induced CR increased for Gr2, and 3 and decreased for Gr4.

Conclusion The results suggest that vasovasostomy performed using a surgical technique that minimizes disruption or damage to VD may have a favorable impact on motility.

Keywords EFS (electrical field stimulation) · Vas anastomosis · Vas approximation · Vas deferens motility · Vasovasostomy

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Introduction

Vasovasostomy is used to address iatrogenic transections of the vas deferens (VD) encountered during surgical procedures in the inguinoscrotal region or to reverse sterilization vasectomies.

Iatrogenic transection of the VD is a rare but a devastating perioperative complication in pediatric patients undergoing surgical procedures in the inguinoscrotal region. Although the exact prevalence is not well-established in the literature, earlier pathological evaluations of indirect hernia sacs revealed the presence of either VD or epididymis in pathological specimens up to 1.6% of cases [1–4]. Patients of younger ages, those undergoing surgery for incarcerated hernia, and those undergoing surgeries performed by non-pediatric surgeons/urologists are more susceptible to such injuries.

Vasectomy reversal is a preferred procedure for 6% of men who undergo vasectomy for contraception and for a smaller percentage of men suffering post-vasectomy pain syndrome [5]. Reestablishing vasal continuity relies on an end-to-end, watertight anastomosis without tension. Different surgical techniques, including macroscopic, microscopic, and robotic-assisted techniques, have been described. It is assumed that microscopic and robotic-assisted techniques have similar patency and pregnancy rates, both of which are superior to the macroscopic technique [6]. In addition, an experimental approximation model for vasovasostomy, described by Ozen et al., has been proposed as an easy, short, and cost-effective option [7].

The consequences of vasovasostomy in terms of VD patency, flow rates, inflammation, granuloma formation, and pregnancy rates are well documented in the literature. However, there is currently no data available regarding the effects of vasovasostomy on VD motility. The aim of this experimental study is to evaluate the effects of different vasovasostomy techniques on VD motility.

Methods

The permission for the experimental study was taken from "Animal Ethics Committee" of Gazi University (IRB Number:01/2011-16). Twenty-four male Wistar-albino rats weighing 220–270 g (mean weight 240 ± 10 g) were included. All animals were housed under standard laboratory conditions with light and dark cycles (14 h/10 h). They allowed free access to water and food. Humane care was given to all animals. They were anesthetized using intramuscular injection of xylazine hydrochloride (5 mg/kg) (Alfazyne, Ege Vet, Turkey), and ketamine hydrochloride (40 mg/kg) (Ketalar, Eczacibasi, Turkey), and were allocated to four groups randomly. There were no statistically significant differences in the weights of rats among groups. Two surgeons of the team performed surgical procedures.

Control group (Gr1): These animals underwent laparotomy following lower midline incision and left-sided VD were harvested with no further intervention.

Microscopic vasovasostomy (Gr2): These animals were operated via left transverse suprascrotal incision. The testis was taken out through this incision and left-sided VD was dissected from surrounding tissues, taking great care not to harm vascular structures. The vas was transected in a transverse fashion and an anastomosis was performed under microscope with 8/0 polypropylene sutures. The testicles were replaced afterwards.

Macroscopic vasovasostomy (Gr3): These animals were operated via left transverse suprascrotal incision. The testis was taken out through this incision and left-sided VD was dissected from surrounding tissues, taking great care not to harm vascular structures. The vas was transected in a transverse fashion and three suture approximation technique using 7/0 polypropylene suture was carried out. The testicles were replaced afterwards.

Vas approximation (Gr4): These animals were operated via left transverse suprascrotal incision. The testis was taken out through this incision and left-sided VD was dissected from surrounding tissues, taking great care not to harm vascular structures. The vas was transected in a transverse fashion and approximated with a technique described by Ozen et al. [7]. To approximate the two halves, one sharp end of a 23G needle was inserted to one vas segment just 5 mm proximal from cut end, advanced within the lumen of the either vas segments and extracted from the other vas segment just 5 mm distal to cut end. 6/0 polypropylene suture was passed through the needle and suture was left in situ as a stent after removing the needle. The either ends of the suture were tied and knotted separately to approximate the vas. The testicles were replaced afterwards.

All left-sided VD were harvested for motility studies 12 weeks after surgery and the rats were sacrificed. The pharmacological evaluation was conducted by a team blinded to the groups. All VD strips were mounted in 20 ml organ baths (containing Krebs–Henseleit solution: 118 nM NaCl, 4.7 nM KCl, 2.5 nM $CaCl_2$, 25 nM $NaHCO_3$, 1.2 nM $MgSO_4$, 1.2 nM KH_2PO_4, 11 nM glucose) with a resting tension of 1 g. The pH of the solution remained 7.4 and the temperature maintained at 37 °C after being bubbled with a gas mixture of 95% O_2 and 5% CO_2. All strips were allowed to equilibrate for 60 min at 1gr tension prior to experimental procedures. Isometric contractions were evoked by electrical field stimulation (EFS) through a platinum electrode for

10 s in every 8 min, with the varying degree of stimulation frequencies (1, 2, 4, 8, 16, 32, 64 Hz), trains of impulses of 1 ms duration for 15 ms with a voltage of 60 V. Stimuli were generated by a stimulator (STPT 03, May Research stimulator, COMMAT Iletisim Co., Ankara, Turkey). EFS evoked responses were recorded via isometric force displacement transducers (FDT 10-A, May IOBS 99, COMMAT Iletisim Co., Ankara, Turkey) connected to an online computer via four channels transducer data acquisition system (MP35B-CE BIOPAC Systems Inc., Santa Barbara, CA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc., Santa Barbara, CA) which also had the capacity to analyze the data.

Exogenous α-β-methylene ATP (Sigma Chemical Co., St Louis, MO) was dissolved in the distilled water, stored at − 20 °C, diluted in Krebs solution to reach the required concentration and applied to VD strips at a concentration of 10^{-6} M and 10^{-5} M.

Exogenous noradrenalin hydrochloride (Sigma Chemical Co., St Louis, MO) was dissolved in the distilled water, stored at − 20 °C, diluted in Krebs solution to reach the required concentration, and applied to VD strips at the concentrations of 10^{-6} M to 10^{-4} M.

The contractions of VD strips after the application of 80 mM KCl were also recorded. All EFS-induced and drug-induced contractions were expressed as the percentage of 80 mM KCl-induced contractions.

The experimental results were expressed as mean ± SEM. The Kolmogorov–Smirnov test was performed to evaluate data for normal distrubution. Kruskal–Wallis test was used to test the differences among groups and Mann–Whitney *U* test was used to test the differences between groups. A *p* value less than 0.05 was considered to be statistically significant.

Results

Contractile response (CR) of VD to the first phase of EFS

No statistically significant changes in CR were observed for Gr2 when compared to Gr1, in terms of first phase of EFS. The first phase of EFS-induced CR with stimulation frequencies was increased for Gr3 when compared to Gr1. Even the changes at low frequencies (1 Hz, 2 Hz, and 4 Hz) were not significant, the increases were statistically significant especially with submaximal and maximal frequencies (8 Hz, 16 Hz, 32 Hz, and 64 Hz). Meanwhile, the first phase of EFS-induced CR with stimulation frequencies was decreased for Gr4 when compared to Gr1. Even the changes at low frequencies (1 Hz, 2 Hz, and 4 Hz) were not significant, the decreases were statistically significant especially with submaximal and maximal frequencies (8 Hz, 16 Hz, 32 Hz, and 64 Hz) (Fig. 1).

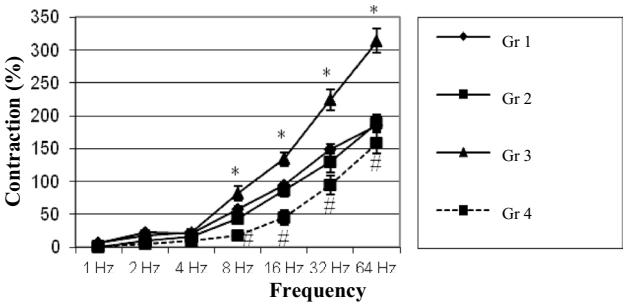

Fig. 1 Effects of microscopic vasovasostomy, macroscopic vasovasostomy, and vas approximation on the first phase of EFS-induced contractile responses of the ipsilateral vas deferens. Frequency response curves were constructed for the vas deferens 12 weeks after the operation. Each symbol represents the mean value ± SEM of the EFS-induced contractions expressed as a percentage of the 80 mM KCl-induced contractions (*$p < 0.05$ for Gr 3 when compared to Gr 1, #$p < 0.05$ for Gr 4 when compared to Gr 1)

CR of VD to the second phase of EFS

The second phase of EFS-induced CR with stimulation frequencies was increased only at the maximal frequency (64 Hz) for Gr2 when compared to Gr1. The second phase of EFS-induced CR with stimulation frequencies was increased for Gr3 when compared to Gr1. Even the changes at low frequencies (1 Hz, 2 Hz, and 4 Hz) were not significant, the increases were statistically significant especially with submaximal and maximal frequencies (8 Hz, 16 Hz, 32 Hz, and 64 Hz). Meanwhile, the second phase of EFS-induced CR with stimulation frequencies was decreased for Gr4 when compared to Gr1. Even the changes at low frequencies (1 Hz, 2 Hz, 4 Hz, and 8 Hz) were not significant, the decreases were statistically significant especially with submaximal and maximal frequencies (16 Hz, 32 Hz, and 64 Hz) (Fig. 2).

CR of VD to exogenous *α*-*β*-methylene ATP

The decreases in CR for Gr3 and Gr4 were statistically significant when compared to Gr1 (Table 1).

CR of VD to exogenous noradrenalin (NA) hydrochloride

The increases in CR for Gr2 and Gr3, and the decrease in CR for Gr4 were statistically significant when compared to Gr1 (Table 1).

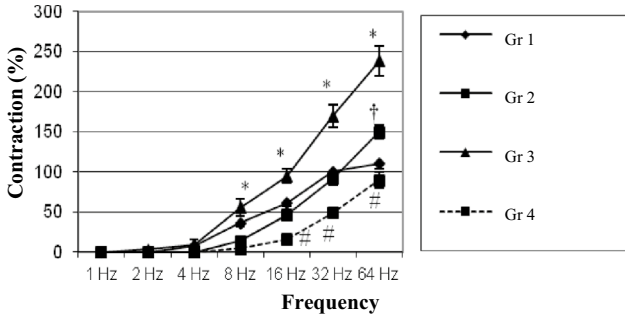

Fig. 2 Effects of microscopic vasovasostomy, macroscopic vasovasostomy, and vas approximation on the second phase of EFS-induced contractile responses of the ipsilateral vas deferens. Frequency response curves were constructed for the vas deferens 12 weeks after the operation. Each symbol represents the mean value ± SEM of the EFS-induced contractions expressed as a percentage of the 80 mM KCl-induced contractions ($^{\dagger}p < 0.05$ for Gr 2 when compared to Gr 1, $^{*}p < 0.05$ for Gr 3 when compared to Gr 1, $^{\#}p < 0.05$ for Gr 4 when compared to Gr 1)

Discussion

The aim of this study was to evaluate the effects of different vasovasostomy techniques on vas motility. The results of the study indicate that vasovasostomy performed using a surgical technique that minimizes disruption or damage to the VD has a significant impact on vasal motility.

VD is a tubular structure that transports sperm from epididymis to the ejaculatory ducts. Its peristaltic activity depends on the thick muscular layer composed of a three layers of smooth muscles (circular in the middle, longitudinal in the inner and outer sides) innervated mainly by adrenergic system and a number of neuromodulating substances [8, 9].

The application of EFS to the rodent vas generates a biphasic contractile activity. The first phase is mediated by adenosine 5′-triphosphate (ATP) released from nerve terminals. It acts on post-synaptic P2X purinoceptors, works as a co-transmitter to NA to form an excitatory postjunctional potential, and creates a fast, transient twitch-like contraction response. The second phase is mediated by synaptic release of NA from the adrenergic nerve endings located within muscular layer. It acts on post-synaptic α_1-adrenoceptors, inducing an activity independent of membrane potential changes and creating a lasting, tonic contraction response [8–10]. Despite the duality of the contraction response, ATP-related twitch-like response seems to be the essential part. Mulyran et al. demonstrated up to a 60% decrease in vas contraction and 90% infertility rates for the mice lacking P2X1 receptors, suggesting that residual NA was not sufficient for ejaculation. The vas of these P2X1-receptor$^{-/-}$ mice neither responded exogenously applied ATP and *α-β*-methylene ATP nor displayed spontaneous and evoked excitatory junctional potentials in vitro [11].

In terms of vas motility, ATP and NA are the primary effectors at the neuromuscular junction. However, it is not certain how these mediators stored and released, most researchers believed that they are stored separately at the nerve endings and released to the synaptic area one by one [8]. EFS mimics a physiological scenario that the mediators are released from the place where they are stored, act on the pre-/post-synaptic receptors and result in a contractile response. Meanwhile exogenous drug applications mimic a scenario where the mediators act on the pre-/post-synaptic receptors at the synaptic area and result in a contractile response.

The findings of our study revealed distinct changes in vas motility for different vasovasostomy techniques. The microscopic vasovasostomy technique showed the most physiological results. The unaffected ATP-related phases (both EFS-induced and exogenous application) and minimally affected NA-related phases (only at maximum frequency of EFS and exogenous application) of vas at the early stages of vasovasostomy were considered significant in terms of motility. At the same time, the findings of macroscopic vasovasostomy technique revealed increased contractile responses at the early stages of vasovasostomy, which can also be acceptable in terms of motility. The decreased contractile responses to exogenous ATP, in contrast to increased contractile responses to both phases of EFS and for exogenous NA application, raised a difficult-to-explain damage. The increased contractile responses to both phases of EFS and for exogenous NA application seemed to be related to augmented neurotransmitter synthesis at the nerve terminal,

Table 1 Maximal contractile responses (E_{max}) for the groups obtained from ipsilateral vas deferens

	Gr 1 ($n=6$)	Gr 2 ($n=6$)	Gr 3 ($n=6$)	Gr 4 ($n=6$)
First phase of EFS	188.98 ± 12.76	189.68 ± 12.27	314.34 ± 18.01*	158.53 ± 15.30$^{\#}$
Second phase of EFS	103.53 ± 9.11	150.13 ± 9.02†	238.76 ± 18.55*	90.02 ± 9.73$^{\#}$
α-β methylene ATP	45.09 ± 3.58	34.84 ± 5.14	33.93 ± 4.70*	30.20 ± 2.71$^{\#}$
Noradrenaline	31.10 ± 1.13	54.88 ± 3.1†	74.38 ± 6.43*	22.97 ± 3.66$^{\#}$

Each value expressed as a percentage of the 80 mM KCl-induced contractions. ($^{\dagger}p < 0.05$ for Gr 2 when compared to Gr 1, $^{*}p < 0.05$ for Gr 3 when compared to Gr 1, $^{\#}p < 0.05$ for Gr 4 when compared to Gr 1)

increased release to the synaptic area, or modified neuronal uptake of the neurotransmitters. Moreover, enhanced receptor sensitivity due to either elevated receptor density or altered receptor signaling pathways could also be possible explanations for the changes. Meanwhile the most plausible explanation for the decreased contractile response of exogenous ATP is the altered balance of purinoreceptor density. Ruen et al. demonstrated the relaxation of smooth muscles by ATP or its end product, adenosine via prejunctional P2Y receptors in an epithelium-dependent manner [12]. Additionally, prejunctional P2Y receptors were shown to inhibit transmitter release from the nerve terminals [13]. Taking these findings together, it can be speculated that the macroscopic vasovasostomy resulted in the augmentation of neurotransmitter synthesis at the nerve terminal, increased release to the synaptic area, and modified neuronal uptake of the neurotransmitters. The findings of vas approximation technique revealed the most pathological results. The decreased ATP and NA-related phases (both EFS-induced and exogenous application) of vas at the early stages of vas approximation were thought to be related to damage, including decreased neurotransmitter release, decreased receptor sensitivity, inhibition of contractile elements, or activation of relaxant pathways.

Both macroscopic and microscopic vas anastomosis techniques are used for vasectomy reversal with similar success rates in human. Despite the higher rates of vas patency (up to 90%) in the literature, achieving pregnancy after surgery occurs in half of men undergoing macroscopic vasovasostomy and up to three quarters undergoing microscopic vasovasostomy [14–16]. This discrepancy could mainly be attributed to the length of time from vasectomy to vasectomy reversal. Lavers et al. demonstrated acute effects of vasectomy are mainly limited to acute inflammation at the site of ligation [17]. As time goes by, chronic inflammation processes take place, and exaggerated immunological responses, formation of antiserum antibodies, or dysfunctions of epididymal epithelium become prominent [18–20]. In animal models, Hamidinia and Wright evaluated the morphological changes of the vas following microscopic vasovasostomy [21–23]. Wright and Hamidinia assessed the effects of vasovasostomy on dogs' vas deferens morphology and demonstrated flattening of the columnar cells, loss of stereocilia, and the presence of interspersed microvilli resulting from luminal dilation due to increased pressure, mainly at the testicular part of the vas deferens [21, 22]. Hamidinia et al. also examined changes in rats and showed full recovery of the mucosa at the 3rd postoperative month, with a consistent finding of marked atrophy in the smooth muscle and luminal dilation at the anastomotic level [23]. In our study, vas continuity was established shortly after vas transection, and vas motility was evaluated 12 weeks after, when the effects of acute inflammation processes were expected to have subsided. Conducting another experimental study to evaluate the long-term effects of vasovasostomy would be intriguing, aiming to determine if these initial findings endure over time, if the damages are permanent, and if they ultimately impact fecundity.

The limitation of our study is the absence of histopathological and/or biochemical analysis of the vas deferens, as the vas strips were used in their entirety to evaluate EFS-induced responses. In addition, we did not have the opportunity to assess the flow rates of the vas or the fecundity of the animals after any injury.

The surgical technique utilized in vasovasostomy appears to have a crucial influence on vas morphology, patency, and future fertility. While the literature extensively documents these well-established outcomes of vasovasostomy, there is currently no available data concerning its impact on VD motility. The results of this study suggest that achieving VD patency through a surgical approach that minimizes disruption or damage to the VD may yield favorable impacts on its motility.

Author contributions AP: contributed to study conception and design, analysis and interpretation of pharmacological data, drafting the article. GSOF: contributed to acquisition, analysis and interpretation of pharmacological data. IOO: contributed to study conception and design, and revision of the article. FI: contributed to acquisition, analysis of pharmacological data. SY: contributed to acquisition, analysis of pharmacological data. SE: contributed to interpretation of pharmacological data. YS: contributed to interpretation of pharmacological data.

Data availability The data that support the findings of this study are available from the corresponding author upon resonable request.

Declarations

Conflict of interest All authors state no conflict of interest about this study.

Approval of research protocol Not applicable.

Informed consent Not applicable.

Registry and the registration number of study Not applicable.

Animal study Yes.

Ethical IRB number 01/2011-16

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