

Short communication

Topical administration of a novel nitric oxide donor, linear polyethylenimine-nitric oxide/nucleophile adduct (DS1), selectively increases vaginal blood flow in anesthetized rats

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The aim of the present study was to test the effects of a topical administration of a novel nitric oxide donor, linear polyethylenimine-nitric oxide/nucleophile adduct (DS1), on vaginal blood flow and hemodynamics in rats. Laser Doppler flowmetry was used to measure blood flow changes following topical application of DS1 (0.3 or 1.5 mg in 0.15 ml saline) into the vagina of anesthetized Wistar rats. *In vivo* hemodynamic parameters were measured with Millar-tip-catheter placed in the left ventricle. DS1 (1.5 mg) increased vaginal blood flow by 191 ± 24 , 226 ± 22 and $166 \pm 23\%$ of the baseline value (at 5, 15 and 30 min, respectively, after application) without affecting systemic blood pressure, heart rate and cardiac function. The increased vaginal blood flow following DS1 application returned to baseline between 45 and 60 min. Thus, topical application of nitric oxide donors such as DS1 may be useful for the treatment of female sexual dysfunction that develops due to an impairment of local blood flow supply to the vaginal tissue.

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Introduction

Female sexual dysfunction (FSD) is an important health issue that affects the quality of life of many women. Four distinct aspect of FSD have been defined, including sexual desire disorders, arousal disorders, orgasmic disorders and sexual pain disorders.¹ The female sexual response cycle is initiated by neurotransmitter-mediated vascular and nonvascular smooth muscle relaxation, resulting in increased pelvic blood flow, vaginal lubrication, and clitoral and labial engorgement.^{2,3} Any disruption in the factors involved in this response may cause sexual dysfunction, notably vasculogenic impair-

ment of the hypogastric-vaginal/clitoral arterial bed.^{1,4}

Several clinical conditions, including atherosclerosis and diabetes mellitus, may be associated with impaired vaginal blood flow and reduced vaginal lubrication, resulting in sexual arousal disorder and dyspareunia.^{1,5} In such conditions, novel therapies aimed at improving vaginal perfusion might be useful to treat FSD. Indeed, a recent study indicated that the nonspecific alpha-adrenergic blocker phentolamine, which enables vascular smooth muscle relaxation, improved vaginal blood flow and vaginal lubrication, resulting in an improved subjective arousal in postmenopausal women with sexual dysfunction.⁶ However, the potential cardiovascular side effects of such therapeutic approach may limit its clinical application, indicating that further efforts are necessary to develop drugs acting selectively on vaginal perfusion. In the present study, we have evaluated the local and systemic effects of a topical application of a slow-releasing nitric oxide donor, linear polyethylenimine-nitric oxide/nucleophile adduct (DS1), on vaginal blood flow assessed by laser Doppler flowmetry, in anesthetized female rats. Our results indicate that DS1 provides rapid and substantial hyperemia to the vagina, without resulting in systemic hypotension or other systemic hemody-

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dynamic effects, suggesting its possible utility in the therapy of FSD related to impaired vaginal blood flow.

Methods

Measurement of vaginal blood flow

Female Wistar rats (300–350 g) were anesthetized with thiopentone sodium (60 mg/kg, i.p.) and intubated to facilitate breathing. Animals were placed on controlled heating pads, and core temperature was measured via a rectal probe maintained at 37°C using a servo-controlled homeothermic blanket. A laser Doppler probe (0.5 mm, Transonic Systems, Ithica, NY, USA) was placed into the distal third of the vaginal vault against the peritoneal surface of the vagina, in order to measure tissue blood flow, as described previously.⁷ The probe was held in place by a holder designed for precision stereotactic neurosurgical placement and was attached to a flowmeter (ALF-21; Transonic Systems, Ithica, NY, USA). The laser Doppler flowmeter was calibrated to an external standard (zeroed) prior to each measurement. Running alongside the laser Doppler flow probe, a 10-cm PE50 tube, ending 2 mm proximal to the distal tip of the Doppler flow probe, was attached to inject the fluid into the vaginal vault. DS1 (0.3 or 1.5 mg) or its backbone, an inactive polymeric compound (1.5 mg), was dissolved in 0.15 ml of 37°C normal saline immediately before use and injected once.

Hemodynamic measurements

Left ventricular function and mean blood pressure were determined in anesthetized rats as described previously.^{7,8,9} Briefly, a microtip catheter transducer (SPR-524; Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle under pressure control. A P50 tube was also inserted into the right femoral artery to measure arterial blood pressure. After stabilization for 30 min, the pressure signal was continuously recorded using a MacLab A/D converter (AD Instruments, Mountain View, CA, USA), and stored and displayed on an Apple Macintosh computer. Heart rate, left ventricular systolic and end-diastolic pressures (LVSP and LVEDP) were measured and the maximal slope of systolic pressure increment ($+dP/dt$) and diastolic pressure decrement ($-dP/dt$), indexes of contractility and relaxation, were calculated. After the end of the experiments, animals were euthanized with an overdose of pentobarbital.

Materials

DS1 was synthesized as previously described.¹⁰

Statistical analysis

Data are expressed as mean \pm s.e.m. Continuous data were evaluated with repeated measures analysis of variance. When the relevant F values were significant at the 5% level, pairwise comparisons were made for the effect of time in specific groups, using Dunnett's test with time 0 as a control, and for the effect of treatment at specific times, using Student–Newmann–Keuls adjustments. A *P*-value of <0.05 was regarded as statistically significant.

Results

As shown in Figure 1, topical administration of DS1 (1.5 mg) into the vaginal vault rapidly increased vaginal blood flow to 191 ± 24 , 226 ± 22 and $166 \pm 23\%$ of the baseline value at 5, 15 and 30 min, respectively, following application ($n=10$). Vaginal blood flow returned to baseline between 45 and 60 min (127 ± 17 and $107 \pm 11\%$ at 45 and 60 min, respectively). Moreover, DS1 (1.5 mg) had no effect on systemic blood pressure, heart rate, LVSP, dP/dt , $-dP/dt$ and LVEDP (Figures 1 and 2). A lower dose of DS1 (0.3 mg) also tended to increase vaginal blood flow, but this effect did not reach statistical significance ($n=12$). The inactive polymer (1.5 mg) exerted no significant effects on vaginal blood flow and measured hemodynamic parameters ($n=8$; Figures 1 and 2).

Discussion

A variety of neuromodulators have been implicated to play a role in clitoral and penile erection as well as lubrication of the vagina during arousal.^{11,12} Nitric oxide (NO) is widely acknowledged to be a major neuromodulator implicated in the initiation and maintenance of penile erection. In fact, a key breakthrough in the therapy of male sexual dysfunction was reached by the introduction of selective cyclic GMP phosphodiesterase inhibitors, which prolong the biological vasodilatory response to endogenously produced NO. Since there are many homologies between male and female sexual tissues, it is conceivable that NO also acts as a neurotransmitter in smooth muscle relaxation in the clitoris and vagina.^{11,12} In humans, NO synthase isoforms are present in clitoral¹³ and vaginal¹⁴ tissues. Basal

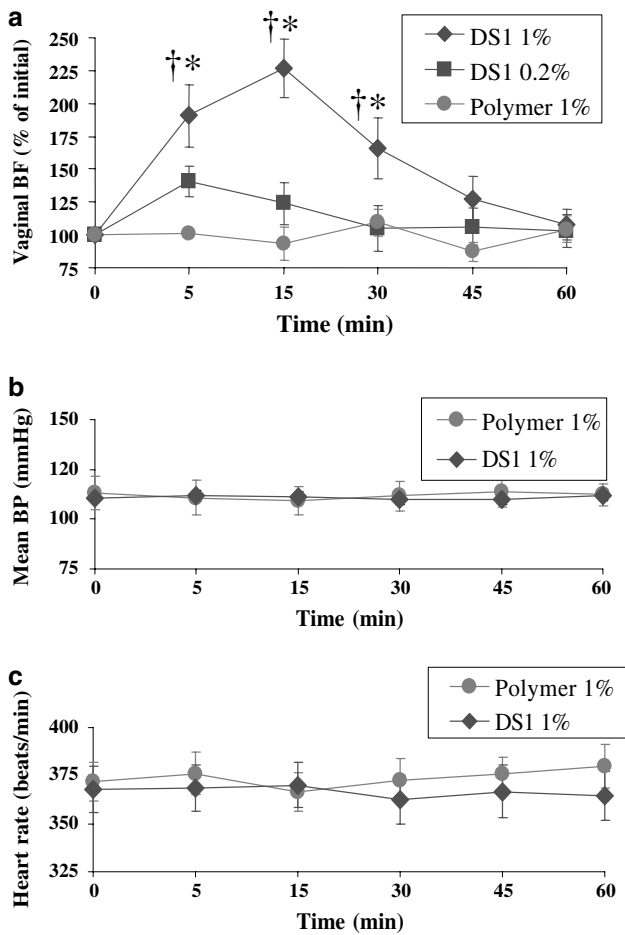


Figure 1 Topical administration of DS1 into the vaginal vault dose-dependently increases vaginal blood flow (BF) without affecting mean blood pressure (BP) and heart rate in anesthetized rats. Results are mean \pm s.e.m. of 8–12 experiments in each group. * $P < 0.05$ vs inactive polymer. † $P < 0.05$ vs baseline.

NO production plays a critical role in the maintenance of basal blood flow to virtually all tissues in the body. Therefore, NO may also play an important role in controlling blood flow in these tissues during sexual arousal.¹¹ Surprisingly, no direct studies have been conducted to determine the role of NO in the maintenance of blood flow to female reproductive tissues. The latter aspect is particularly relevant to the pathophysiology of FSD, considering that vaginal engorgement and clitoral erection critically depend on increased local blood inflow.¹⁵

We have assessed the effects of a novel NO donor, DS1, on vaginal blood flow in anesthetized female rats. DS1 belongs to the category of NO/nucleophile adduct vasodilators, which do not require biological tissue activation for NO release, and release NO spontaneously in aqueous solutions. As reported previously, approximately 30% of the weight of DS1 is NO, which is slowly and spontaneously released from the core of the molecule, with a half-life of NO

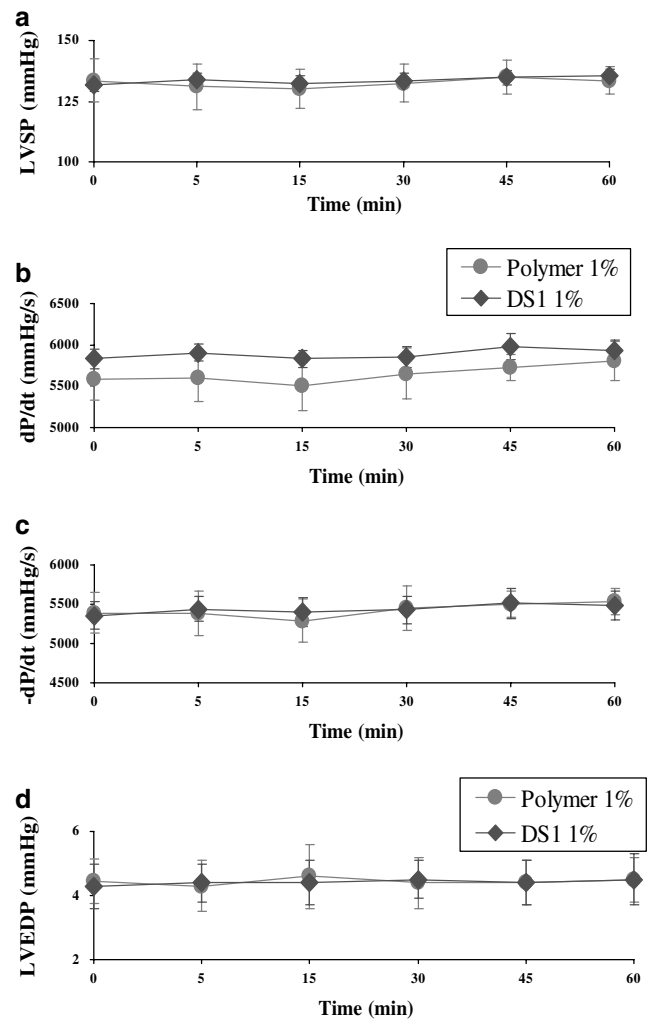


Figure 2 Topical administration of DS1 into the vaginal vault has no effects on LVSP, LVEDP, left ventricular +dP/dt and -dP/dt. Results are mean \pm s.e.m. of 8–10 experiments in each group.

release of approximately 2 h at 37°C.¹⁰ Although we did not measure it directly, we assume that DS1 and/or NO released from DS1 rapidly penetrate the vaginal mucosa and lead to a local vasodilatation. It is well known that NO is a rapidly acting vasodilator, which relaxes the vascular smooth muscle via a cGMP-dependent mechanism. As discussed previously,¹⁰ the vasodilator effect of NO/nucleophile adducts (as opposed to various nitrovasodilators) is not associated with desensitization or tolerance development, which is likely to increase their utility as rapidly acting topical vasodilators.

In the present study, we tested the effect of DS1 *in vivo* using a laser Doppler perfusion monitoring (LPDM). Previous investigators have shown that LPDM represents a suitable technique to assess vaginal vasculogenic events occurring with electrical stimulation of the pelvic nerves in the female rat.^{7,15,16} LPDM signal is an index of the amount of blood present and going through the site of tissue

illuminated by the laser beam. As such, LPDM represents a useful tool to study the physiology, pharmacology and sexual dysfunction relating to blood flow in vaginal tissue.⁷

The major new finding of the present study is that the topical administration of the NO donor DS1 into the vaginal vault rapidly and selectively increases vaginal blood flow for more than 30 min (Figure 1). It is noteworthy that the vaginal wall engorgement with blood plays a central role in genital sexual arousal, being a critical mechanism enabling lubrication of the inner surface of the vagina. In a recent study evaluating vaginal physiological changes in a model of sexual arousal in the rat, Giuliano *et al*¹⁶ reported that pelvic nerve stimulation induced a rapid and transient increase in vaginal blood flow (assessed by LPDM), followed by an elevation in vaginal temperature and partial pressure in oxygen. The magnitude of the effect of DS1 on vaginal blood flow in our study is comparable to that observed by Giuliano *et al*,¹⁶ indicating that the DS1-triggered increase in vaginal perfusion mimics the typical physiological changes noted during genital sexual arousal. Importantly, the effects of DS1 were strictly restricted to the vagina, and we did not notice any systemic effects of this compound, as demonstrated by the lack of influence of DS1 on arterial blood pressure (Figure 1) and physiological parameters of cardiac function (Figure 2). These findings are in agreement with our recent study showing that inhalation of aerosolized DS1 produces selective pulmonary vasodilation without adversely affecting the systemic circulation.¹⁰ Thus, DS1 appears to be a local NO releaser that is free from distant vasodilatory side effects.

As more clinical and basic science studies are dedicated to the problem of female sexual dysfunction, it is likely that novel therapies for this disorder will be developed in the years to come. In addition to hormone replacement therapy, vasodilating compounds are regarded as promising therapeutic agents, including phentolamine, sildenafil and prostaglandin E₁.^{1,6} In addition to these agents, our current study suggests that topical application of a slow-releasing topical NO donor such as DS1 selectively increases vaginal blood flow, with the great advantage of leaving the systemic cardiovascular status unaffected. Therefore, this strategy may be particularly well suited for the treatment of

female sexual dysfunction relating to blood flow disturbances in vaginal tissue.

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