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Impact of topic administration of nitric oxide donor gel in the clitoridian blood flow, assessed by Doppler ultra-sound☆

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A B S T R A C T

Objective: This study aims to evaluate of the impact of NO molecules in Pluronic F-127 gel, applied topically in the clitoris, on the clitoridian blood flow of healthy volunteer women, using the Doppler ultra-sound.
Method: A total of 20 healthy women over 18 years old and sexually active with no sexual hormones alteration were enrolled. The Doppler ultra-sound procedure was performed on the artery of the clitoris in patients without the NO donor gel, and then after fifteen minutes of its application the same procedure was done again, to compare the values.
Results: The hemodynamic results showed that this formulation was responsible for the increase of the systolic and diastolic speeds by about 2.5 times after 15min of the administration of the gel. The initial resistance index was increased by 1.2 due to the local venous congestion in only 15min after the administration of gel, indicating that this product can be used to promote the dilatation of the artery of the clitoris to treat women with sexual dysfunction.
Conclusion: The use of topic hydrogel as a donor drug in the clitoris of women resulting in a local vasodilatation, without systemic effects. These findings suggest that this preparation may be useful in the management of selected cases of female sexual dysfunction.

Impacto en la administración de un donante de óxido nítrico en gel en el flujo sanguíneo clitoridiano, medido por eco-Doppler

Palabras clave:
Pico máximo de velocidad de flujo sistólica y diastólica
Índice de resistencia
Eco-Doppler

R E S U M E N

Objetivo: Evaluar el impacto de las moléculas del donante de óxido nítrico (NO) en el gel F-127 plurónico, aplicado tópicamente en el clítoris, sobre el flujo sanguíneo clitoridiano de mujeres jóvenes voluntarias, usando eco-Doppler.
Método: Veinte mujeres sanas mayores de edad, sexualmente activas y sin alteraciones sexuales en su historia clínica fueron enroladas para la medición mediante eco-

☆Winning project of the award of the International Society for Sexual Medicine 2008.

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Clítoris
Flujo sanguíneo

Doppler de la velocidad del pico sistólico, velocidad diastólica y tasa de resistencia en las arterias clitoridianas previo a la aplicación del donante del NO en gel, a fin de observar los valores basales del flujo en ellas y comparar los valores pre y post.
Resultados: Las velocidades sistólica, diastólica y resistencia de base, y 15 min posterior a la aplicación del gel, fueron significativamente diferentes (p = 0,002, p = 0,043, p = 0,005, respectivamente). Los resultados muestran el aumento de las velocidades sistólicas y diastólicas a nivel de las arterias clitoridianas en casi 2,5 veces después de 15 min de la administración del gel, lo que traduce un aumento en el flujo sanguíneo clitoridiano.
Conclusión: El uso del donante de NO en gel S-nitrosoglutation aumenta, significativamente, el flujo sanguíneo del clítoris. Esto podría ser usado terapéuticamente en algunos casos de disfunción sexual.

Introduction

Nitric oxide, whose chemical structure is NO, is a soluble, highly lipophilic gas synthesized by endothelial cells, macrophages, and some neurons. NO is a very important intracellular and extracellular mediator. It also induces the enzyme guanylate cyclase (GC), producing cyclic guanosine monophosphate (cGMP) responsible for smooth muscle relaxation, which causes biological actions such as vasodilation and bronchodilation.[1]

NO is one of the smallest and most simple molecules synthesized.[2] It is a colorless, inorganic gas with no free radicals having seven electrons derived from nitrogen and eight electrons derived from oxygen plus an odd electron. As with some intestinal peptides, NO is involved in the process of clitoral tissue swelling during sexual stimulation.[3]

NO has been shown to be synthesized by nitric oxide synthetase (NOS), an enzyme of paramount importance for protecting blood vessels.[4]

Since it was first described as an endothelial-derived relaxing factor in 1987, NO has been considered as one of the most important biochemical messengers. It has many different functions and is distributed throughout the human body.

Despite its apparent chemical simplicity, NO is considered as a promising therapeutic agent. Many therapeutic problems are prevented when NO-derived gels are topically administered.[5]

NO is known to be important for vasodilation of the cavernous body in males and clitoris in females, and cavernous body relaxation problems are known potential causes of sexual dysfunction.

According to the World Health Organization, sexual dysfunction is a serious health problem because it impairs quality of life. Sexual dysfunction has been reported in 30%-50% of women in the United States.

According to the US census, more than nine million women report discomfort during intercourse and difficulty in reaching orgasm.

The potential significance of vascular mechanisms in the pathophysiology of sexual stimulation and orgasm has led to the study of the effects of drugs to facilitate the early phases of sexual arousal. Such drugs included oxytocin, which may increase sexual receptiveness, but was not used for sexual dysfunction because no excipient is available for its release. The clitoris is also known to have a significant role in this phase.[6]

This study was intended to assess the impact of NO molecules in the Pluronic F-127 gel, topically applied to the clitoris, on clitoral blood flow of healthy female volunteers using Doppler ultrasound.

Patients and methods

This was a prospective study approved by the local ethics committee in which 20 sexually active, healthy women were enrolled. The study was conducted at the division of outpatient female urology of Universidade Estadual de Campinas (Brazil).

Inclusion criteria

The following subjects were considered eligible for the study:

a. Women over 18 years of age.
b. Women with no sex hormone changes, as shown by serum tests (estrogens and free and total testosterone).
c. Women with no history of sexual disorders.

Exclusion criteria

The following subjects were excluded from the study:

a. Pregnant women.
b. Women with past or current sexual dysfunction.
c. Women with current urinary or vaginal infection.
d. Postmenopausal women.
e. Women with significant vaginal prolapse identified in physical examination.

f. Women with diabetes or any other disease possibly causing changes in the peripheral nerve system.
g. Women using drugs that could interfere with the peripheral nerve system.

Clinical assessment

Clinical history, physical examination, hormone assessment, and Doppler ultrasound of clitoral arteries were performed for patient selection at the baseline visit.

Synthesis of nitric oxide donor

The S-nitrosoglutathione (GSNO) derivative was selected as nitric oxide donor. GSNO was synthesized by reaction of equimolar amounts of glutathione with sodium nitrate in an aqueous hydrochloric acid solution (0.5M HCl), mixed in an ice container for 40 min.[7] The solution was precipitated with acetone, filtered, and subsequently washed with water and acetone again. Precipitation was freeze-dried for 24 h. GSNO was kept at the freezer (-20ºC) and protected from light.

Preparation of gel containing nitric oxide donor

Pluronic F-127 (25% by weight) in water contains GSNO (100 µM) and was prepared as reported elsewhere.[7] Solid pluronic F-127 was added to cold water (5ºC). This solution was kept at 5ºC for 12 hours to achieve a balanced polymer solution. Aqueous GSNO solution (0.35 mM) was added to pluronic F-127 under stirring in an ice container until fully homogeneous.

Doppler ultrasound of clitoral arteries

This phase started with a pilot study intended to assess the time required for the NO derivative to reach its peak action. This was assessed by topical administration of the gel to patients, followed by Doppler ultrasound examination and observation of hemodynamic variables from the start of gel administration to their peak values, occurring approximately 15 minutes later. The dose of NO donor in the gel used for this study was 100 µM, and the product was synthesized by the chemistry institute of our institution.

A baseline Doppler ultrasound examination of clitoral arteries was performed before application of the NO donor gel to record baseline flow values. Hemodynamic parameters assessed included peak systolic velocity, diastolic velocity, and resistive index. One milliliter of gel, containing a 100 µM dose, was subsequently applied onto the clitoris, and Doppler ultrasound measurements were again performed after 15 minutes to compare the values. A 7.5 MHz Toshiba transducer was used for translabial ultrasonography. All Doppler ultrasound measurements were performed by the same investigator using the same procedure.

Variables and concepts

Maximum velocity (Vmax) is the peak arterial systolic velocity.

Minimum velocity (Vmin) is diastolic velocity.

Resistive index (RI) is resistance to blood flow in the vessel. RI is calculated using the formula:

$$IR = \frac{Vmax–Vmin}{Vmax}$$

Statistical analysis

A Wilcoxon test was used for statistical analysis. The significance level used was 5% ($p<0.05$).

Results

After Doppler ultrasound, an analysis of systolic velocity at baseline and 15 min after gel application showed significant differences between them (p=0.002) (fig. 1).

Diastolic flow velocities found in both measurements were markedly different (p=0.043) (fig. 2).

A comparison of resistance at baseline and after gel administration also showed a significant difference (p=0.005) (fig. 3).

Figure 4 shows blood flow in the clitoris before use of the gel. In figure 5, decreased vessel resistance is the result of local vasodilation caused by the gel 10 min after administration, while figure 6 shows dilation of clitoral arteries at 15 min, represented by resistance.

Discussion

Medical literature shows that the NO donor molecule, GSNO, incorporated into the pluronic F-127 gel caused local vasodilation when administered into the clitoral arteries of patients.

Pluronic F-127 was selected as excipient to be incorporated into GSNO because it is a biologically tested polymer used in medical formulations such as treatments for burns, rectal administrations, etc.[5]

At low temperatures (below 10ºC) pluronic F-127 is an isotropic solution at all concentrations. Above 20ºC, micelles reacommodate in a cubic structure and the solution becomes a hydrogel.

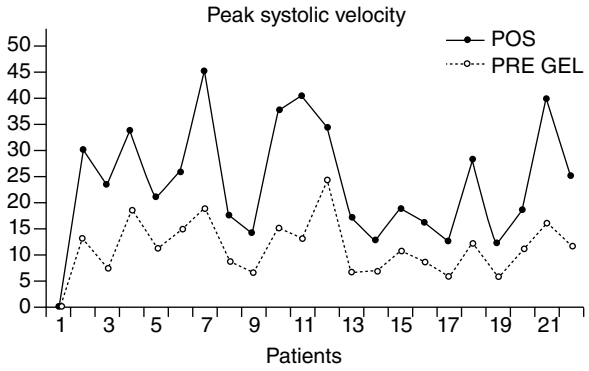

Figure 1 – Assessment of peak systolic flow.

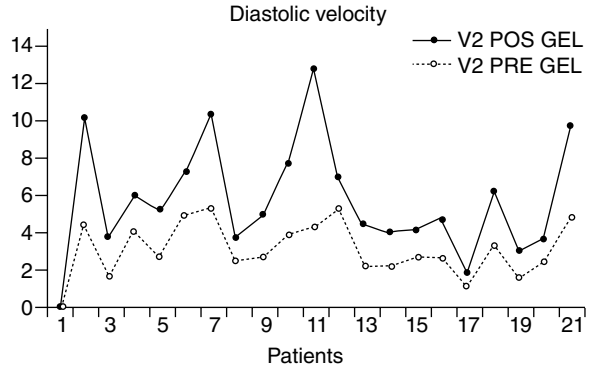

Figure 2 – Assessment of peak diastolic flow.

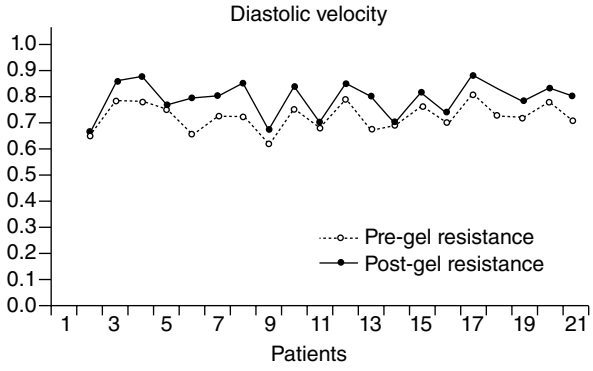

Figure 3 – Resistive index.

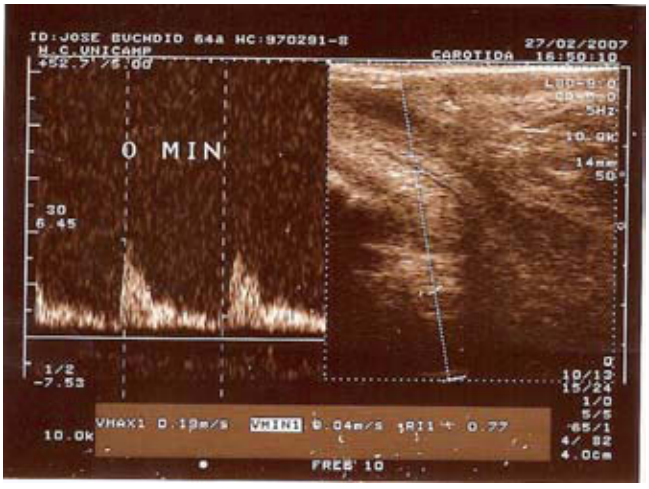

Figure 4 – Doppler ultrasound before administration of NO donor gel showing a resistive index in the vessel = 0.77.

Topical use of hydrogel as a derived drug allows for extensive release of GSNO into the microcirculation, resulting in local vasodilation without systemic effects.[5]

Peak hemodynamic effect occurs after 15 min of administration. As a hydrophilic molecule, GSNO may readily diffuse through plasma and intracellular matrix to cause direct release of GC.

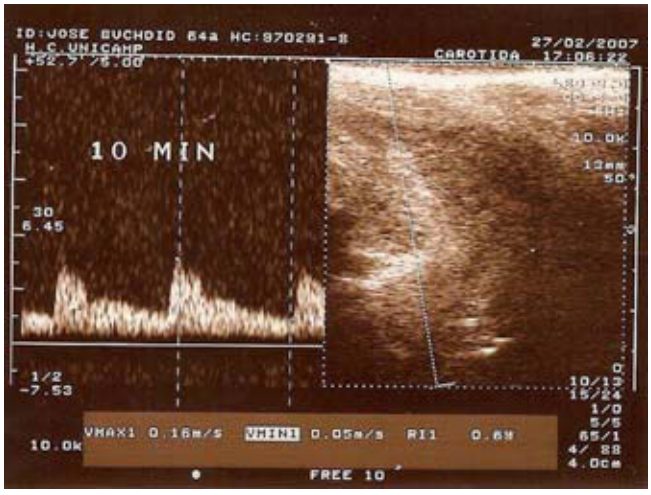

Figure 5 – Doppler ultrasound 10 min after gel administration showing a decreased resistance: 0.69.

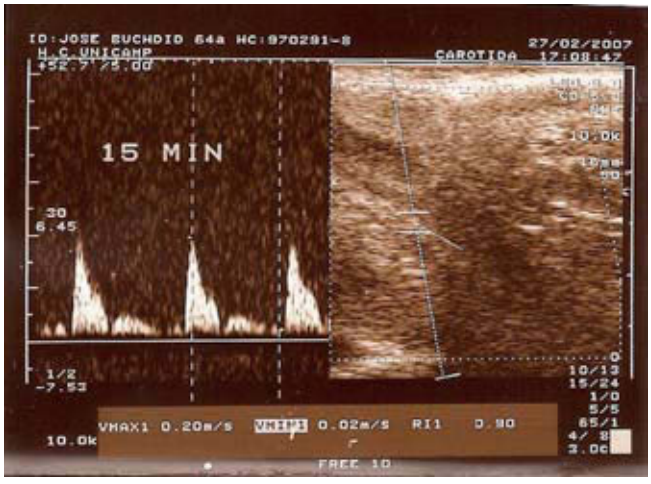

Figure 6 – Doppler ultrasound 15 min after gel administration, showing an increase in resistance to 0.90 due to local venous congestion.

NO produced by endothelial cells is essential in the relaxation process of blood vessels. Under physiological conditions, relaxation occurs when cell membrane receptors are activated by stimuli from soluble molecules including acetylcholine, bradykinin, adenosine diphosphate, substance P, serotonin, etc. or when attrition of circulating cells on endothelial layers increases, activating the NOS enzyme (e-NOS), which produces NO.[8]

e-NOS is strategically bound to the endothelial cell membrane, promoting the presence of great amounts of NO close to the muscle layer of blood vessels and cells. In response to agonists such as bradykinin, e-NOS is phosphorylated and translocated to the cytosol.[9]

NO produced at endothelial cells is moved to muscle cells and vessel lumen. The rapid diffusion of this molecule and its easy penetration into other cells because of its small size and lipophilicity are important for understanding its biological activities.[10] Within muscle cells, NO interacts with iron in

the GC enzyme, which alters conformation of the enzyme, activating it (aGC). The aGC catalyzes exit of two phosphate groups from the guanosine triphosphate molecule, resulting in cGMP. Increased cGMP levels in muscle cells causes muscle relaxation.[11]

There are many potential applications of NO, but doubts still exist about its therapeutic indications. Most recent *in vivo* data suggest that its use may be beneficial in healing wounds caused by diabetes, skin infections, local vasodilation in diabetes, and psoriasis.[12]

Sildenafil citrate was recently shown to be effective for treating erectile dysfunction in males. Sildenafil exerts its action through inhibition of the enzyme 5-phosphodiesterase, which prevents degradation of cGMP. It thus promotes high cGMP levels, relaxing smooth muscles of the cavernous body of the penis, promoting erection and maintaining it for a longer time.[13]

Use of sildenafil in women with sexual dysfunction has not been successful, partly because of the inclusion criteria.[14]

This study showed a lower resistance as compared to baseline after gel use due to local vasodilation caused by this substance. Increased blood flow in clitoral arteries similarly accounts for the subsequent increase in resistance in such arteries due to swelling of the clitoris.

The lack of a corneal layer in vaginal mucosa allows for GSNO diffusion into a specific area. Hemodynamic results showed for the first time that this formulation increased systolic and diastolic velocities by almost 2.5 times 15 min after gel administration. RI increased 1.2 times after administration as compared to baseline due to local venous congestion. These results showed that an increased clitoral blood flow may be achieved as early as 15 min after administration, which suggests that this product may be used to promote dilation of clitoral arteries and cavernous bodies.

These findings suggest that the preparation tested may be of value in management of selected cases of female sexual dysfunction.

Conclusion

Use of the NO donor in GSNO significantly increases blood flow in the clitoris. This effect may be used for therapeutic purposes in some cases of sexual dysfunction.

Conflict of interest

Authors state that they have no conflicts of interest.

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