HHS Public Access

Author manuscript

Int J Impot Res. Author manuscript; available in PMC 2022 September 21.

Published in final edited form as:

Int J Impot Res. 2022 September ; 34(6): 573–580. doi:10.1038/s41443-021-00451-6.

Topically delivered nitric oxide acts synergistically with an orally administered PDE5 inhibitor in eliciting an erectile response in a rat model of radical prostatectomy.

Moses T. Tar[1], **Joel M. Friedman**[2], **Andrew Draganski**[3], **Kelvin P. Davies**[1,2,*]

[1]Department of Urology, Albert Einstein College of Medicine, Bronx, NY, USA.

[2]Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, USA.

[3]Zylo Therapeutics, Inc., Greenville, SC, USA

Abstract

Patients undergoing radical prostatectomy (RP) have a high incidence of post-operative erectile dysfunction (ED) refractory to treatment by oral phosphodiesterase-type-5-inhibitors (PDE5i). In the present studies, we investigated if a topically applied, nitric oxide microparticle delivery system (NO-MP) might act synergistically with an oral PDE5i (sildenafil) to improve erectile function outcomes in a rat model of RP.

Thirty-five Sprague–Dawley rats underwent bilateral transection of the cavernous nerve (CN) for one week. After one-week, animals were orally administered 0, 0.05 or 0.005 mg sildenafil/kg and the erectile response following topical application to the penile shaft of 250mg or 100mg NO-MP, or blank-MP, was monitored over a two-hour timeframe by recording the intracorporal pressure normalized to systemic blood pressure (ICP/BP, N=5 animals/treatment group).

Oral treatment with sildenafil by itself resulted in no observable erectile response. However, a combination of orally administered 0.05 sildenafil/kg with topical application of 250mg NO-MP, compared to 250 mg NO-MP by itself, resulted in significantly more spontaneous erections (4.6 compared to 2 erections per hour, t-test; p-value = 0.043), with a significantly faster onset for the first erectile response (11 compared to 22 minutes; t-test, p-value = 0.041).

Our results demonstrate a synergistic effect between orally administered PDE5i and topically applied NO-MP in eliciting an erectile response. Furthermore, they suggest a potential novel therapeutic approach to treat men with ED resulting from RP, through combination therapy of a topically applied NO-MP and an orally administered PDE5i.

*Corresponding Author: Kelvin P. Davies, Ph.D., Department of Urology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA; Phone: 718 430 2914; Fax: 718 430 3201; kelvin.davies@einsteinmed.org.

Conflict of interest: The authors declare that they have no conflict of interest.

Keywords

erectile dysfunction; radical prostatectomy; cavernous nerve injury; phosphodiesterase-type-5 inhibitor; nitric oxide; topical delivery; microparticle

Introduction.

Data from the National Cancer Institute (www.cancer.gov) shows that prostate cancer (PrCa) is the second most common cancer in men. In 2018, there were an estimated 1,276,106 new cases of PrCa worldwide, with more than one-tenth of these cases occurring in the USA[1]. The most commonly used therapeutic approach to treat patients with localized prostate cancer is radical prostatectomy (RP). Although the long term oncologic outcomes following RP are good, and with nerve-sparing and robotic assisted RP there is increasing PrCa survivorship, a significant number of patients suffer from post-surgical erectile dysfunction (ED), which has a severe impact on their health and well-being[2–4]. A recent meta-analysis suggests even with the use of nerve-sparing robotic surgical procedures, erectile function outcomes after RP are not significantly improved[5].

The release of nitric oxide (NO) from cavernous nerve (CN) endings is known to play a key role in initiating an erectile response[6,7]. NO activates cyclic guanosine monophosphate (cGMP) synthase, generating the secondary messenger cGMP, which ultimately results in cavernosal smooth muscle relaxation and vascular inflow[8,9]. The FDA approved first-line oral treatments for ED (sildenafil, tadalafil, avanafil and vardenafil) all act as phosphodiesterase-5 inhibitors (PDE5i)[10]. They target the enzyme PDE5 to prevent the break-down of cGMP, in effect enhancing the erectile response pathways activated by NO[11]. A primary cause of ED following RP is believed to result from iatrogenic CN injury leading to the loss of NO mediated activation of pathways involved in erectile function[7,12]. As a result, the majority of patients undergoing RP suffer from ED that is refractory to treatment through the use of PDE5i[13].

Recognizing that the loss of NO signaling due to CN injury plays a significant role in the development of ED after RP, in past preclinical studies we have demonstrated the potential of a topically applied microparticle NO delivery system (NO-MP) to generate an erectile response in an animal models of RP[14,15]. Given that the mechanism of action PDE5i relies on NO activating erectile response pathways, there is a strong rationale to expect a synergistic response between treatments that increase local NO levels and PDE5i in eliciting an erection. Therefore, in the present studies we investigated the potential synergy between NO-MP and an orally administered PDE5i (sildenafil) in eliciting an erectile response in a rat model of RP.

Materials and Methods.

Animal model of radical prostatectomy.

The use of animals in the present study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and was approved by the Animal Care and Use Committee of Albert

Einstein College of Medicine. A total of 35 male Sprague–Dawley rats, 4–5 months old (weighing on average ~275 g) were obtained from Charles River Laboratories (Boston, MA, USA) and underwent bi-lateral transection of the CN (as previously described[14]) as a model of nerve injury that occurs during RP. Briefly, the procedure involves performing a vertical lower abdominal midline incision, identifying the major pelvic ganglion (MPG) on either side of the dorsolateral lobes of the prostate (which has two inputs, the hypogastric nerve and the pelvic nerve, with the CN being one of its outputs). CN transection was achieved by directly cutting the nerve 2–5 mm distal to the MPG under dissecting microscope visualization. After one-week, erectile responses in animal were determined visually and through measurement of the intracorporal pressure (ICP) (normalized to blood pressure (BP)).

Microparticle Preparation.

The NO-MP was produced by Zylo Therapeutic, Inc. (Greenville, SC, USA) by a modification of procedures outlined in a previous publication[16]. Briefly, the process consists of (i) acid-catalyzed hydrolysis of two silicate precursors, tetraethylorthosilicate (TEOS) and mercaptopropyltrimethoxysilicate (MPTS); (ii) co-condensation of hydrolyzed TEOS and MPTS to form a highly porous thiolated sol-gel monolith; (iii) a series of proprietary steps to remove any unreacted monomer and nano-sized particulate matter; and (iv) a drying step to evaporate residual water and ethanol. Its use has been previously described in several prior publications[16,17]. Prior to application to the rat penile shaft, the thiol groups of the NO-MP were nitrosated by combining with equimolar parts of sodium nitrite and glycolic acid in a partially aqueous vehicle. This facilitates the release of NO, which lasts up to several hours.

Determination of erectile response following treatments:

We have previously described the procedures to determine ICP and BP[14,18]. Rats were treated 30 minutes before being anesthetized for surgery by oral gavage of 0 (sildenafil negative control), 0.05 or 0.005 mg sildenafil/kg (Sigma-Aldrich Corp., St. Louis, MO, USA)/kg (equivalent to clinical doses of approximately 2.5 and 0.25 mg sildenafil for an 80 kg patient, respectively[19]). Rats were anesthetized via intraperitoneal injection of sodium pentobarbital (35 mg/kg, Henry Schein, Inc, Melville, NY, USA) and a cannula was inserted into the carotid artery for determination of systemic BP. Next an incision was made in the perineum, the ischiocavernosus muscle removed to expose the corpus cavernosum crus, and a 23-gauge needle inserted to measure ICP. The changes in ICP and systemic BP were monitored continuously throughout the experiments, as previously described[14]. After establishing baseline ICP and BP for 30 min, sildenafil negative control animals and animals receiving 0.05 sildenafil/kg, were treated with 250 or 100 mg NO-MP, or non-nitrosated, blank-MP formulation through application to the rat penile shaft (N=5 animals per group, for a total of 30 animals). Animals receiving 0.005 sildenafil/kg were treated only with 250 mg NO-MP (N=5). Erectile response was then monitored erectile over the approximately 2-hour time course of the experiment. Animals were studied for the time taken for an initial erectile response, duration of the erectile response, the maximal and average ICP/BP response observed and the number of spontaneous erections per hour.

Statistical Analysis and Rigor.

A difference of at least 0.2 in ICP/BP was considered biologically important. Based on prior studies, to achieve 80% statistical power, and a significance level $\alpha=0.05$ a difference in ICP/BP ≥0.2 could be determined by comparing five animals in each experimental group. Animals were randomly assigned into each experimental group. The investigator performing the measurement of ICP/BP was blinded as to whether they were using the NO-MP or blank-MP. No animals were excluded from the data analysis unless they suffered pathology as a result of the surgical procedures.

Data are expressed as mean and standard error of the mean (S.E.M). A modified t-test was used to compare erectile responses (time taken for an initial erectile response, duration of the erectile response, the maximal and average ICP/BP response observed and the number of spontaneous erections per hour) between treatment groups. A value of $P<0.05$ was considered statistically significant.

Results.

Topically applied microparticles delivering nitric oxide (NO-MP) elicit an erectile response in an animal model of RP.

A typical experimental result and trace of the ICP/BP response following topical application of the control (blank-MP) or NO-MP is shown in Fig. 1. Whereas application of blank-MP resulted in no erectile response over the approximately 2 hours duration of the experiment (Fig. 1A), application of NO-MP resulted in an erectile response after approximately 35 min (an increase in the ICP/BP ratio >0.6, corresponding to a visible erection (Fig. 1B)).

Table 1 shows the characteristics of the erectile response for each treatment group. No erectile response was observed in the 30 minutes prior to topical application of the test formulation, and no erectile response was noted in any control animals treated with blank-MP over the entire 2 hour duration of the experiment. On average, animals treated with 250 mg NO-MP demonstrated an initial erectile response after 22 minutes, 2 erections per hour, with a maximum ICP/BP of 0.42 and duration of 6.5 minutes, whereas animals receiving 100 mg NO-MP demonstrated an initial erectile response after 63 minutes, 1 erection per hour, with a maximum ICP/BP of 0.27 and duration of 6 minutes.

No animal had systemic BP or heart rate outside of normal Sprague-Dawley rat parameters, and there were no significant differences between control and experimental groups (BP ranged from 140–170 cmH_2O; 103–125 mmHg[20] and heart rate from 330–480 heart beats per minute[21]). Following determination of erectile function, animals were sacrificed with subsequent gross necropsy revealing no obvious differences between control and NO-MP treatment.

Topically applied microparticles delivering nitric oxide (NO-MP) act synergistically with orally administered sildenafil to elicit an erectile response in an animal model of RP.

A typical experimental ICP/BP trace following orally administered 0.05 mg sildenafil/kg, with or without subsequent topical application of 250 mg NO-MP after 50 minutes is shown

in Fig. 2. Application of oral sildenafil alone resulted in no erectile response over the 2 hours duration of the experiment (Fig. 2A), whereas subsequent topical application of 250 mg NO-MP formulation resulted in an erectile response after approximately 10 minutes with several spontaneous erections observed over the course of an hour.

Table 2 shows the characteristics of the erectile response for each treatment group. In animals pre-treated 0.05 mg sildenafil/kg followed by topical application of 250 mg NO-MP (i) the time for the first erection to develop took approximately 11 minutes, significantly faster than the 22 minutes in the absence of sildenafil (Table 1), (ii) 4.6 erections occurred per hour, significantly more than the 2 erections per hour in the absence of sildenafil (Table 1), (iii) average maximum ICP/BP response was 0.41 with a duration of 8.5 minutes, values that were not significantly different to the absence of sildenafil (Table 1).

In animals pretreated with 0.05 mg sildenafil/kg followed by topical application of 100 mg NO-MP or pre-treated with 0.005 mg sildenafil/kg followed by topical application of 250 mg NO-MP the measured parameters for the erectile response were not significantly different to the values in animals that were not pretreated with sildenafil (Table 1).

Fig. 3 summarizes the major synergistic effects of orally administered sildenafil followed by topical application of NO-MP, which the time for the first erectile response and the erections per hour. Again, no animals where sildenafil was orally administered had systemic BP or heart rate outside of normal parameters and there were no significant differences between control and experimental groups. Following determination of erectile function, animals were sacrificed with subsequent gross necropsy revealing no obvious differences between control and NO-MP treatment.

Discussion.

The results presented here add to observations reported in previous studies that topical application of an NO-MP formulation is able to elicit an erectile response in an animal model of radical prostatectomy[14]. Furthermore, we demonstrate a synergistic effect between an orally administered PDE5i (sildenafil) and topical application of NO-MP on this erectile response. Knowledge of the molecular pathways that lead to an erection provide a rationale for this synergistic effect (as depicted in Fig. 4)[11]. At present, we cannot distinguish which cells represent the major site of action of NO released from the NO-NP (i.e. endothelial cells to produce more NO, or smooth muscle to cells to activate the cGMP synthase, or both cells types). However, raising NO levels in cavernosal tissue would activate cGMP synthase in smooth muscle cells, thereby increasing cGMP levels, which in turn activates erectile response pathways. The presence of the PDE5i, sildenafil, would inhibit the breakdown of cGMP, and therefore be expected to act synergistically with NO in generating a greater, and more prolonged, activation of erectile response pathways.

The NO-MP used in these formulations although generated by a similar process as previously described and used in published studies[14,15,17,18,22,23], have modifications that would facilitate clinical translation. In the NO-MP formulations used in the present studies, NO is stored via nitrosation of thiol groups, which greatly reduces the potential toxicity that

might occur from residual sodium nitrite that is a contaminant in the synthesis of the original NO-MP formulations[16]. In addition, the silicate precursor tetramethylorthosilicate (TMOS) was replaced by TEOS in the synthesis. This is an important when considering scale-up of the fabrication process, as TMOS is a highly reactive ingredient that becomes increasingly dangerous to handle, whereas TEOS is much less reactive and can be handled with far greater safety. The resulting second-generation NO-MP used in these studies is a highly porous nitrosothiolated silicate powder with a size distribution of 1–20 μm. The formulation is “non-gritty”, small enough to be readily absorbed into the stratum corneum but without the concerns of deeper penetration into the viable dermis and potentially incorporation into systemic circulation. The NO-MP, once applied to the skin, will deliver an initial burst of NO followed by logarithmic decay over 30 minutes for a total yield of 3 μmole NO/mg of particles. Assuming efficient delivery to corporal smooth muscle cells, this capacity is well within the predicted amounts of NO needed to generate an erection: an analysis of nitrate and nitrite elevation in corporal tissue after stimulation suggests that ~5 nmole of NO is generated during erection in a rat penis (weighing on average 250 mg)[24].

The main synergistic effects of combining orally administered sildenafil and topical application of NO-MP were a decrease in the time-for-onset of the first erectile response and an increase in the number of spontaneous erections per hour. A faster response time is clinically relevant as a major complaint of men who do experience a positive response through the use or oral PDE5i is the lag-period prior to effect[25]. In addition, the effective concentration of sildenafil needed to produce an erectile response when used in combination with topically applied NO-MP was equivalent to a clinical dose of 2.5 mg, far lower than the lowest commercially available sildenafil dose (25 mg from Pfizer). Therefore, topical application of NO-NP could significantly reduce the effective oral dose of PDE5i, mitigating some of the associated side-effects. Men are also routinely prescribed low dose PDE5i for “penile rehabilitation” following RP (although this is ineffective for treating ED), and also low daily doses of tadalafil are prescribed as an alternative to “on demand” treatment for ED[26]. The synergistic effect demonstrated in this research between an orally administered PDE5i followed by topical application of NO-MP potentially could potentially facilitate a greater efficacy for these groups of patients.

For clinical translation it is important to consider the limitations of this animal model of RP. CN architecture is less complex in the rat than in humans. In addition, the rat penile dermis is significantly thinner, and therefore presents less of a barrier for penetration of NO-MP than in humans. Additional studies are planned with human cadaver skin to determine, and if necessary, optimize, dermal penetration by the NO-MP. It would also be necessary for patients to activate the NO-MP by mixing with glycolate just prior to use. In order facilitate this we have developed a prototype two-compartment single-use package solution in which end-user simply breaks the frangible seal and mixes the two components; this mixing causes the nitrosation reaction to occur, thereby “activating” the NO-MP. However, the underlying molecular pathways involved in the erectile response are the same between rats and humans, and would support a rationale in patients that undergo RP to have ED treated by combination of an orally administered PDE5i and topically delivered of NO.

In conclusion, we demonstrate that topical application of a microparticle particle formulation delivering NO (NO-MP) can generate an erectile response in an animal model of RP and provide the first reported evidence that topically delivered NO can act synergistically with orally administered PDE5i to increase the erectile response. Overall, our results suggest novel treatment strategies for ED in patients who have undergone RP and are therefore refractory to oral PDE5i and also to potentially increase the efficacy and safety of PDE5i for all patients being treated for ED.

Acknowledgments.

Thank-you to Scott Pancoast and Lisa Lallos for suggestions to improve the manuscript.

Funding:

This work was supported by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant R41 DK121587, R01 DK109314 and Grant P20 DK123969].

Abbreviations:

BP	blood pressure
cGMP	cyclic guanosine monophosphate
CN	cavernous nerve
NO	nitric oxide
NO-MP	NO-releasing microparticles
PDE5i	phosphodiesterase-type-5 inhibitor
PrCa	prostate cancer
ICP	intracorporal pressure
MPG	major pelvic ganglion
MPTS	mercaptopropyltrimethoxysilicate
TEOS	tetraethylorthosilicate

References.

1. Rawla P Epidemiology of Prostate Cancer. World J Oncol 2019; 10(2): 63–89. [PubMed: 31068988]
2. McCullough AR. Sexual Dysfunction after Radical Prostatectomy. Reviews in Urology 2005; 7(Suppl 2): S3–S10.
3. Shabsigh R, Anastasiadis AG. Erectile dysfunction. Annu Rev Med 2003; 54: 153–168. [PubMed: 12525671]
4. Mallis D, Moisidis K, Kirana PS, Papaharitou S, Simos G, Hatzichristou D. Moderate and severe erectile dysfunction equally affects life satisfaction. J Sex Med 2006; 3(3): 442–449. [PubMed: 16681469]
5. Coughlin GD, Yaxley JW, Chambers SK, Occhipinti S, Samaratunga H, Zajdlewicz L et al. Robot-assisted laparoscopic prostatectomy versus open radical retropubic prostatectomy: 24-month

outcomes from a randomised controlled study. Lancet Oncol 2018; 19(8): 1051–1060. [PubMed: 30017351]

6. Burnett AL, Musicki B. The nitric oxide signaling pathway in the penis. Curr Pharm Des 2005; 11(31): 3987–3994. [PubMed: 16378505]
7. Sezen SF, Lagoda G, Burnett AL. Neuronal nitric oxide signaling regulates erection recovery after cavernous nerve injury. J Urol 2012; 187(2): 757–763. [PubMed: 22177198]
8. Ghalayini IF. Nitric oxide-cyclic GMP pathway with some emphasis on cavernosal contractility. Int J Impot Res 2004; 16(6): 459–469. [PubMed: 15229623]
9. Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun 1990; 170(2): 843–850. [PubMed: 2166511]
10. Huang SA, Lie JD. Phosphodiesterase-5 (PDE5) Inhibitors In the Management of Erectile Dysfunction. P T 2013; 38(7): 407–419. [PubMed: 24049429]
11. Andersson KE. PDE5 inhibitors - pharmacology and clinical applications 20 years after sildenafil discovery. Br J Pharmacol 2018; 175(13): 2554–2565. [PubMed: 29667180]
12. Selvin E, Burnett AL, Platz EA. Prevalence and risk factors for erectile dysfunction in the US. Am J Med 2007; 120(2): 151–157. [PubMed: 17275456]
13. Padma-Nathan H, McCullough AR, Levine LA, Lipshultz LI, Siegel R, Montorsi F et al. Randomized, double-blind, placebo-controlled study of postoperative nightly sildenafil citrate for the prevention of erectile dysfunction after bilateral nerve-sparing radical prostatectomy. Int J Impot Res 2008; 20(5): 479–486. [PubMed: 18650827]
14. Tar M, Cabrales P, Navati M, Adler B, Nacharaju P, Friedman AJ et al. Topically applied NO-releasing nanoparticles can increase intracorporal pressure and elicit spontaneous erections in a rat model of radical prostatectomy. J Sex Med 2014; 11(12): 2903–2914. [PubMed: 25302850]
15. Han G, Tar M, Kuppam DS, Friedman A, Melman A, Friedman J et al. Nanoparticles as a novel delivery vehicle for therapeutics targeting erectile dysfunction. J Sex Med 2010; 7(1 Pt 1): 224–233. [PubMed: 19765204]
16. Abuzeid WM, Girish VM, Fastenberg JH, Draganski AR, Lee AY, Nosanchuk JD et al. Nitric oxide-releasing microparticles as a potent antimicrobial therapeutic against chronic rhinosinusitis bacterial isolates. Int Forum Allergy Rhinol 2018; 8(10): 1190–1198. [PubMed: 30044542]
17. Yang C, Mavelli GV, Nacharaju P, Li K, Cleare LG, Nosanchuk JD et al. Novel nitric oxide-generating platform using manuka honey as an anti-biofilm strategy in chronic rhinosinusitis. Int Forum Allergy Rhinol 2020; 10(2): 223–232. [PubMed: 31834677]
18. Draganski A, Tar MT, Villegas G, Friedman JM, Davies KP. Topically Applied Curcumin-Loaded Nanoparticles Treat Erectile Dysfunction in a Rat Model of Type-2 Diabetes. J Sex Med 2018; 15(5): 645–653. [PubMed: 29699754]
19. Gur S, Sikka SC, Knight GE, Burnstock G, Hellstrom WJ. Purinergic contraction of the rat vas deferens in L-NAME-induced hypertension: effect of sildenafil. Asian J Androl 2010; 12(3): 415–421. [PubMed: 20305675]
20. Redfors B, Shao Y, Omerovic E. Influence of anesthetic agent, depth of anesthesia and body temperature on cardiovascular functional parameters in the rat. Lab Anim 2014; 48(1): 6–14. [PubMed: 23985835]
21. Azar T, Sharp J, Lawson D. Heart rates of male and female Sprague-Dawley and spontaneously hypertensive rats housed singly or in groups. J Am Assoc Lab Anim Sci 2011; 50(2): 175–184. [PubMed: 21439210]
22. Karakus S, Musicki B, Navati MS, Friedman JM, Davies KP, Burnett AL. NO-Releasing Nanoparticles Ameliorate Detrusor Overactivity in Transgenic Sickle Cell Mice via Restored NO/ROCK Signaling. J Pharmacol Exp Ther 2020; 373(2): 214–219. [PubMed: 32144123]
23. Friedman AJ, Han G, Navati MS, Chacko M, Gunther L, Alfieri A et al. Sustained release nitric oxide releasing nanoparticles: characterization of a novel delivery platform based on nitrite containing hydrogel/glass composites. Nitric Oxide 2008; 19(1): 12–20. [PubMed: 18457680]
24. Ushiyama M, Kuramochi T, Katayama S. Treatment with hypotensive agents affects the impaired relaxation of the penile corpus cavernosum in hypertensive rats. Hypertens Res 2006; 29(7): 523–532. [PubMed: 17044665]

25. Zucchi A, Costantini E, Scroppo FI, Silvani M, Kopa Z, Illiano E et al. The first-generation phosphodiesterase 5 inhibitors and their pharmacokinetic issue. Andrology 2019; 7(6): 804–817. [PubMed: 31350821]

26. Mulhall JP, Brock G, Oelke M, Fode M, Probst KA, Henneges C et al. Effects of Tadalafil Once-Daily or On-Demand vs Placebo on Return to Baseline Erectile Function After Bilateral Nerve-Sparing Radical Prostatectomy--Results from a Randomized Controlled Trial (REACTT). J Sex Med 2016; 13(4): 679–683. [PubMed: 27045264]

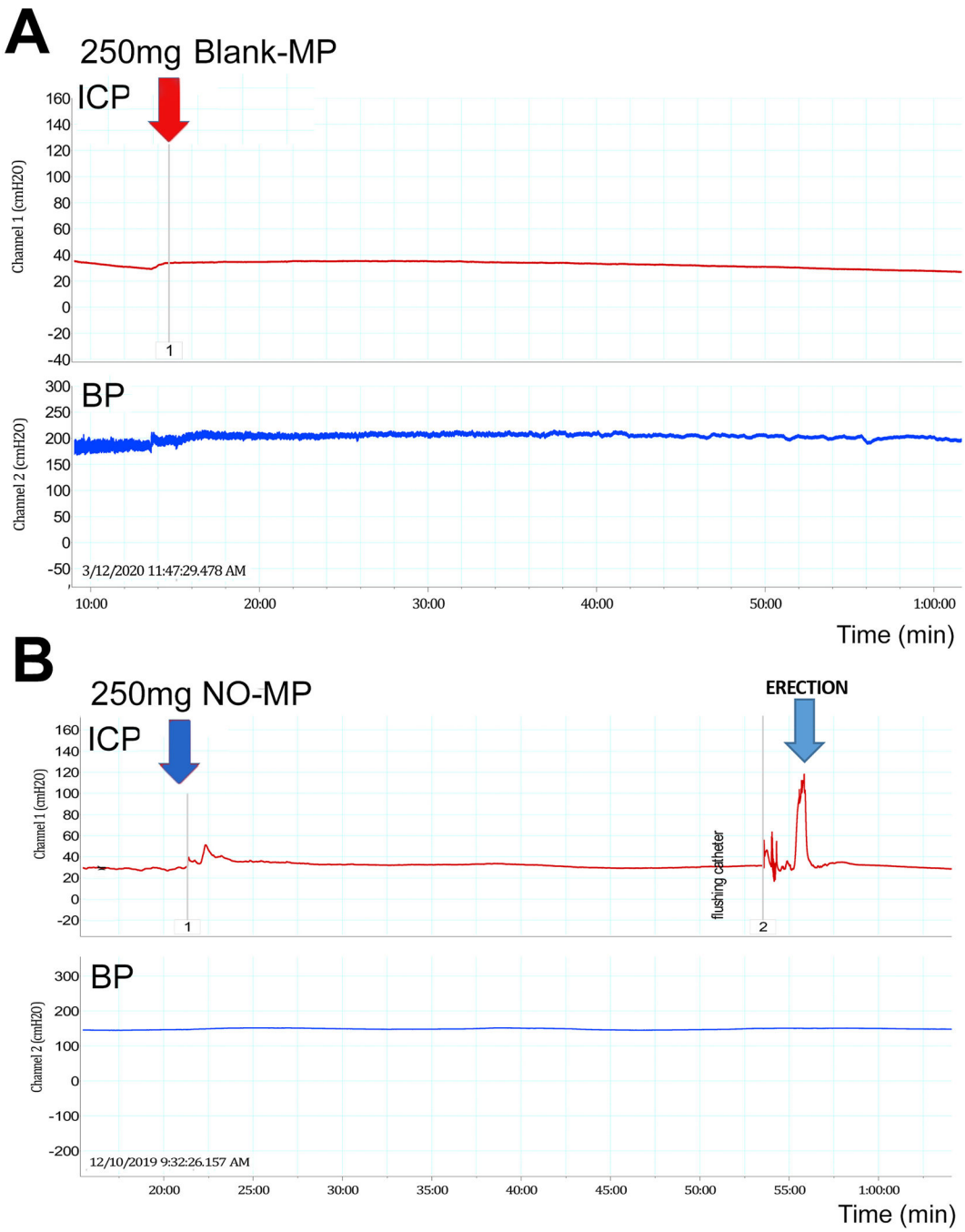

Figure 1A:

Representative data trace for the effect of topical application of 250mg of blank-MP to the penile shaft of a rat that underwent CN transection one week earlier. Upper panel shows intracorporal pressure (ICP) (cmH_20) over time. Lower panel shows systemic blood pressure (BP) (cmH_20) over time.

Figure 1B: Representative data trace for the effect of topical application of 250mg of NO-MP to the penile shaft of a rat that underwent CN transection one week earlier. Upper

panel shows intracorporal pressure (ICP) (cmH_20) over time. Lower panel shows systemic blood pressure (BP) (cmH_20) over time.

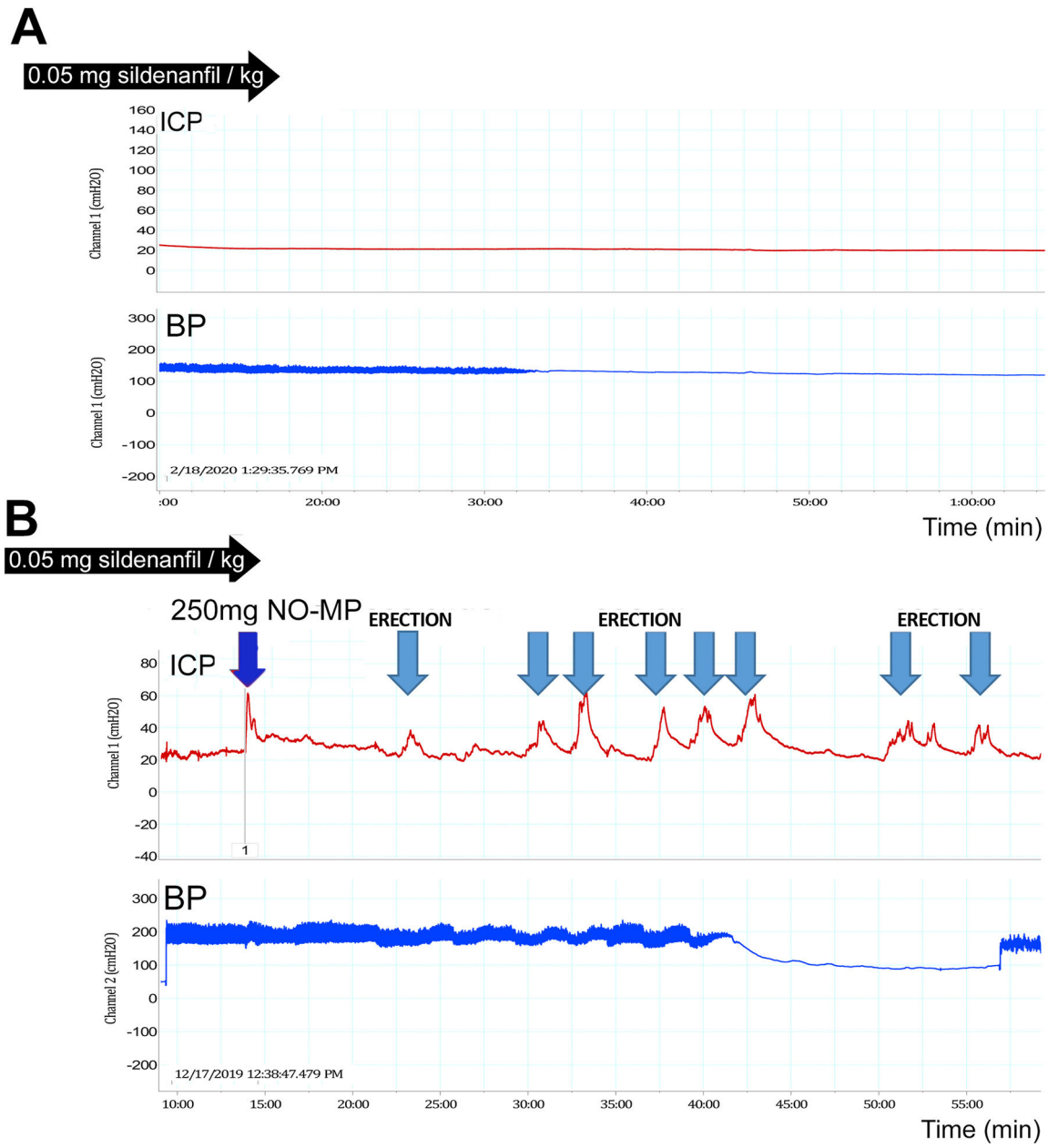

Figure 2A:

Representative data trace for the effect of oral administration of 0.05 mg sildenafil/kg 30 minutes prior to the determination of intracorporal pressure (ICP) and systemic blood pressure (BP) in a rat that underwent CN transection one week earlier. Upper panel shows intracorporal pressure (ICP) (cmH_20) over time. Lower panel shows systemic blood pressure (BP) (cmH_20) over time. (Note: 0.05 mg sildenafil/kg is equivalent to a clinical dose of 2.5mg given to an 80 kg man)

Figure 2B: Representative data trace for the effect of oral administration of 0.05 mg sildenafil/kg 30 minutes prior to the determination of intracorporal pressure (ICP) and systemic blood pressure (BP) in a rat that underwent CN transection one week earlier. Approximately 50 minutes after administration of sildenafil of 250mg of NO-MP to the

penile shaft Upper panel shows intracorporal pressure (ICP) (cmH_20) over time. Lower panel shows systemic blood pressure (BP) (cmH_20) over time.

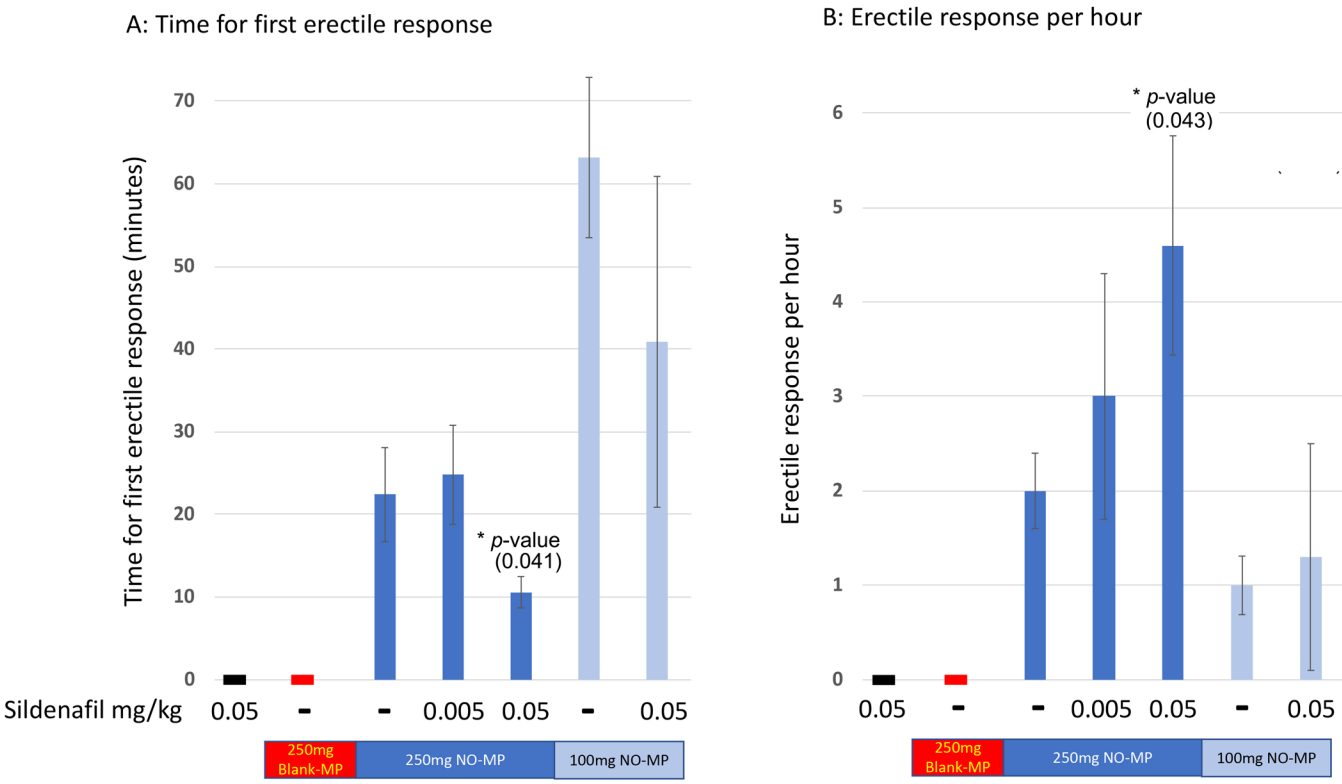

Figure 3.

The major synergistic effects of orally administered sildenafil followed by topical application of NO-MP on A) the time for the first erectile response and B) the erections per hour. Number of animals per group is reported in Tables 1 and 2. *=significant difference ($P<0.05$) between animals receiving orally administered sildenafil used in combination with 250 mg NO-MP compared to animals treated with 250 mg NO-MP but no orally administered sildenafil. In animals treated with 0.05 mg sildenafil/kg alone or 250 mg blank-MP alone there was no erectile response for the duration of the experiment. Note: 0.05 mg sildenafil/kg is equivalent to a clinical dose of 2.5mg given to an 80 kg man, 0.005 mg sildenafil/kg is equivalent to a clinical dose of 0.25mg given to an 80 kg man.

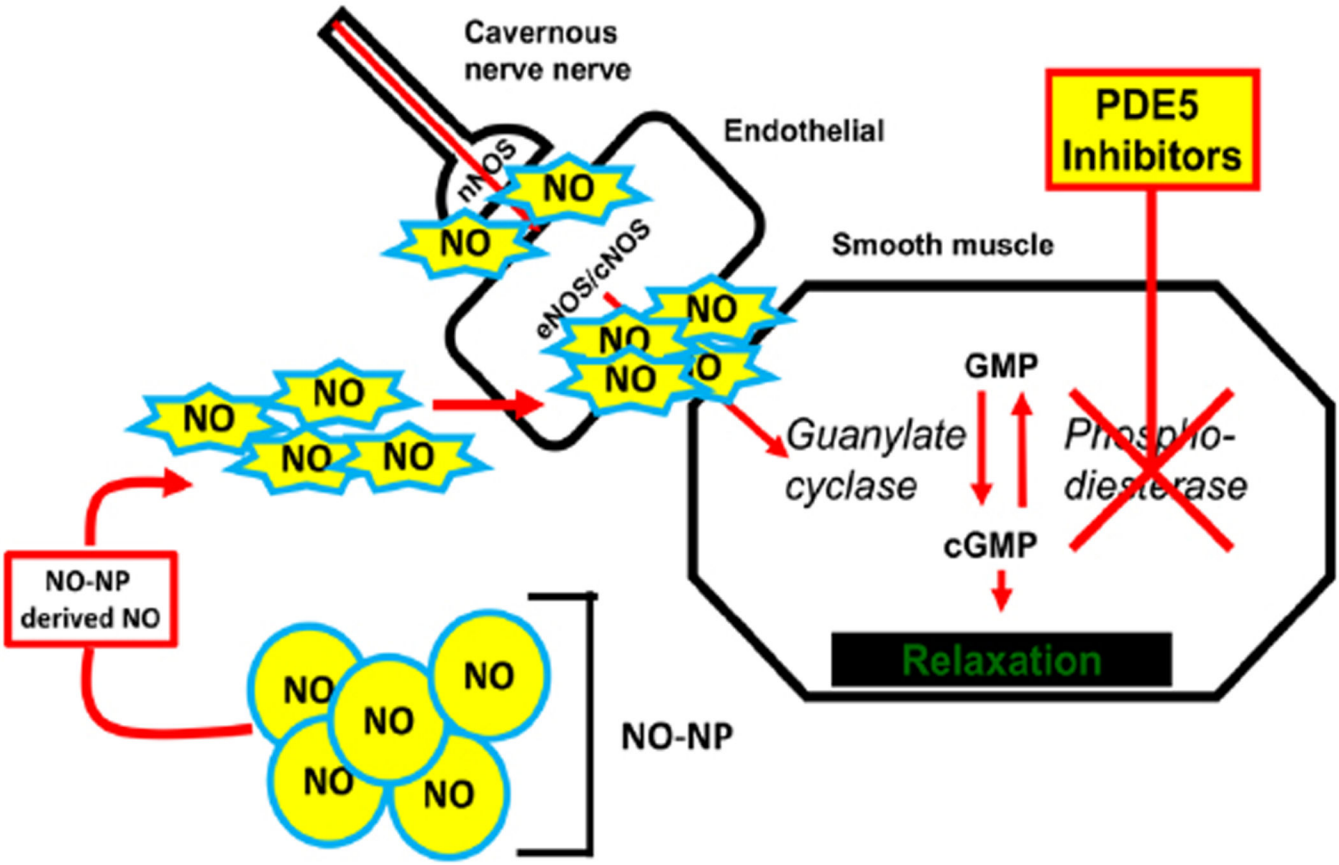

Figure 4.

Depicts the rationale for the studies presented here investigating synergy between treatments that elevate local NO penile tissue levels and inhibit PDE5 in eliciting an erectile response. Topical application of the NO-MP raises local concentrations of NO in corporal tissues which initiates an erection through activation of cyclic guanosine monophosphate (cGMP) synthase. PDE5i then prevents the breakdown of cGMP potentially prolonging and increasing the activation of downstream biochemical pathways resulting in an erection.

Table 1:

Averaged erectile response characteristics for each treatment group.

Treatment (N)	Average Baseline ICP/BP (SEM)	Average BP duration of experiment (SEM)	Average Maximum ICP/BP (SEM) (*p*-value)	Maximum ICP/BP (range)	Time to first erection (minutes) (SEM) (*p*-value)	Duration of erection (minutes) (SEM) (*p*-value)	Erections per hour (SEM) (*p*-value)
250mg Blank-MP (N=3)	0.16 (0.006)	140.6 (13.8)	-[#]		-[#]	-[#]	-[#]
250mg NO-MP (N=5)	0.17 (0.017)	142.1 (15.8)	**0.48 (0.1)** **(0.0024)** *a*	0.19–0.74	**22.4 (5.7)** **(0.0034)** *a*	6.5 (2.2)	**2 (0.4)** **(0.019)** *a*
100mg NO-MP (N=5)	0.12 (0.009)	159.4 (7.2)	**0.27 (0.03)** **(0.0024)** *a*	0.23–0.33	**63.26 (9.7)** **(0.0034)** *a*	6.0 (0.59)	**1.0 (0.31)** **(0.019)** *a*

= No erectile response was observed in animals treated with the Blank-MP (Average Maximum ICP/BP was not significantly different to Average Baseline ICP/BP).

a = a significant difference between the 100mg and 250mg NO-MP treatment groups (*t*-test, *p*-value < 0.05).

Table 2:

Averaged erectile response characteristics for each treatment group.

Treatment (*N*)	Average Baseline ICP/BP (SEM)	Average BP duration of experiment (SEM)	Average Maximum ICP/BP (SEM) (*p*-value)	Maximum ICP/BP (range)	Time to first erection (minutes)(SEM) (*p*-value)	Duration of erection (minutes) (SEM) (*p*-value)	Erections per hour (SEM) (*p*-value)
0.05 mg sildenafil/kg (no NO-MP) (N=4)	0.19 (0.05)	140.6 (13.8)	-#		-#	-#	-#
0.05 mg sildenafil/kg plus 250 mg NO-MP (N=5)	0.15 (0.02)	154.9 (10.7)	**0.41 (0.09)** **(0.010)** *a*	0.19–0.65	**10.6 (1.9)** **(0.0035)** *a*	8.5 (4.4)	**4.6 (1.16)** **(0.013)** *a*
0.05 mg sildenafil/kg plus 100 mg NO-MP (N=5)	0.18 (0.03)	168.6 (8.6)	**0.29 (0.06)** **(0.010)** *a*	0.13–0.44	**38.9 (7.6)** **(0.0035)** *a* **(0.05)** *b*	9.1 (3.5)	**1.3 (1.2)** **(0.013)** *a*
0.005 mg sildenafil/kg plus 250 mg NO-MP (N=5)	0.18 (0.05)	145.4 (14.9)	0.38 (0.07)	0.18–0.58	**24.8 (6.0)** *b* **(0.05)** *b*	4.8 (2.4)	3.0 (1.3)

(Note: 0.05 mg sildenafil/kg is equivalent to a clinical dose of 2.5mg given to an 80 kg man, 0.005 mg sildenafil/kg is equivalent to a clinical dose of 0.25mg given to an 80 kg man).

= No erectile response was observed in animals treated with 0.05 mg sildenafil/kg only (Average Maximum ICP/BP was not significantly different to Average Baseline ICP/BP).

a = a significant difference between the 0.05 mg sildenafil/kg plus 250 mg NO-MP and the 0.05 mg sildenafil/kg plus 100 mg NO-MP treatment groups (*t*-test, *p*-value < 0.05).

b = a significant difference between the 0.05 mg sildenafil/kg plus 250 mg NO-MP and the 0.005 mg sildenafil/kg plus 100 mg NO-MP treatment groups (*t*-test, *p*-value < 0.05). There were no significant between the 0.05 mg sildenafil/kg plus 100 mg NO-MP and the 0.005 mg sildenafil/kg plus 250 mg NO-MP treatment groups.