고신대학교 의과대학 학술지 제21권 제2호
Kosin Medical Journal
Vol. 21. No. 2, pp. 139~144, 2006

동물모델에서 PDE5 억제제의 투여경로에 따른 효과비교

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Phoshodiestrase 5 inhibitors according to the route of administration in the animal model

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Abstract

Background : Intracavernosal (IC) PDE5 (phosphodiesterase type 5) inhibitors, at tissue levels approaching millimolar concentrations, have been reported to cause penile erection in the absence of sexual stimulation. The objectives of this study were to confirm the effects of three PDE5 inhibitors on penile erection as IC agents, without PNS (pelvic nerve stimulation), and compare the efficacy of these agents on penile erection after intravenous (IV) administration with PNS to that of IC administration without PNS.

Materials and Methods : Anesthetized male New Zealand White rabbits (3.5-4.0 kg) were divided into two groups, and the penile hemodynamics was assessed by mornitoring of the intracavernosal pressure (ICP) after penile erection induced by PNS (2.5 Hz, 10 V, 0.8 msec for 30 seconds) with IV administration of 3 PDE5 inhibitors or IC administration without PNS. ICP recordings were normalized to the systemic systolic arterial pressure (SAP). The intracavernosally or intravenously administered doses of zaprinast, sildenafil and vardenafil were 30-1,000 μg/kg, 1-30 μg/kg and 1-30 μg/kg, respectively.

Results : PNS with IV administration of 3 PDE5 inhibitors caused significant frequency-dependent increase in the ICP. The IC administration of zaprinast, sildnafil and vardenafil produced significant increased in the ICP, in a dose dependent fashion. The ICP/SAPs with highest doses of zaprinast, sildenafil and vardenafil tested were 0.656, 0.550, 0.654 in group with IV administration and 0.625, 0.609, 0.666 in group with IC administration without PNS, respectively.

Conclusion : All 3 PDE5 inhibitors showed increases in the ICP/SAP, in dose dependent manners, in response to IC administration in the absence of PNS as well as PNS with IV administration. IC administration was a much more effective route for penile erection than IV administration.

Key words : Phosphodiesterase inhibitor, PDE5, Erectile dysfunction

Introduction

PDE5 (phosphodiesterase type 5) inhibitors competitively inhibits the hydrolysis of cGMP to GMP by PDE because of structural similarity to cGMP. Zaprinast was developed to mimic the structure of cGMP, and a more selective inhibitor of PDEs (i.e. PDE 1, 5 and 6) than old non-selective PDE inhibitors[1]. And It was reported that its administration coupled with PNS and NO donor had a synergic effect of smooth muscle relaxation in vivo and in vitro.[2] Extensive clinical trial data have firmly established sildenafil as a highly effective and safe agent for erectile dysfunction from diverse etielolgies and sildenafil received

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FDA approval in 1998. Vadenafil is a potent and selective PDE5 inhibitor, which, compared with sildenafil, has been shown to be 10 times more potent on a molar basis as a PDE5 inhibitor and more selective for PDE5 over PDE6 and PDE11 in vivo study[3]. It had been accepted that in the absence of sexual stimulation, these drugs have no effect on penile erection. To whether these agents produce an erection in the absence of sexual stimulation, Rosen et al[4] reported that these agents did not initiate or produce an erection in the absence of sexual stimulation with consequent nitric oxide release, and Bischoff and Schneider[5] reported that high dose PDE5 inhibitors cause penile erection without sexual stimulation. Nowadays, it is reported that PDE5 inhibitors, in nanomolar serum levels, facilatates penile erection only during sexual stimulation, but intracavernosal PDE5 inhibitors, at tissue levles approaching millimolar concentrations, can cause penile erection in the absence of sexual stimulation.[6] Thus, this study was undertaken to comfirm the effects of intracavernosal administration of 3 PDE5 inhibitors (zaprinast, sildenafil and vardenafil) without PNS and intravenous administration with PNS on erectile response in rabbit model and to compare the effects of 3 PDE5 inhibitors for on penile erection according to the route of administration.

Methods

1. Animals

Male New Zealand white rabbits (3.5-4.0 Kg) were sedated with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) and placed in the supine position. Anesthesia was maintained as need with intravenous (IV) sodium pentobarbital (50 mg/ml). A 3cm midline neck incision was fashioned to access the carotid artery. A 20-gauge angiocatheter was introduced into the carotid artery and connected to a PT300 pressure transducers (Model PT300 Grass Instruments/Astromed, Inc., Warwick, RI) to continuously monitor systemic arterial pressure (SAP). Body temperature was maintained using an electric heat pad.

2. Pelvic Nerve Stimulation (PNS)

A 4 cm lower midline abdominal incision was performed to expose the pelvic nerve, which can be identified on the posterolateral aspect of the rectum. Bladder contents were aspirated through the bladder wall with an 18-gauge needle and a 50-ml syringe. Under direct vision, a bipolar platinum wire electrode was hooked onto the pelvic nerve without cutting the nerve. Unilateral PNS was accomplished with a Grass S9 stimulator set at normal polarity and repeat mode to generate a 30-seconds train of square waves with a 10-V pulse amplitude, an 0.8-ms pulse width, and suboptimal frequencies (2.5 Hz). The interval between stimulations was 3-5 minutes, which did not produce nerve exhaustion until after 30 minutes in the control group, as determined by peak amplitude of repeated stimulations.

3. Drugs

Sildenafil citrate and vardenafil HCl were a generous gift from Dr Erwin Bischoff (Bayer AG, Wuppertal, Germany). All other drugs and reagents were obtained from commercially available sources.

4. Intracavernosal Pressure Monitoring (ICP)

The skin overlying the penis was incised, and the corpora cavernosa were exposed at the root of the penis. A 23-gauge needle, filled with 4 U/mL heparin solution and connected to PE-50 tubing, was inserted into the left corpus cavernosum for pressure recording. All pressure measurements were recorded by means of Grass PT-300 pressure transducers connected to PI-1-ACDC signal conditioner modules and a Grass 7400 physiological recorder (Astre-Med Inc., Warwick, RI).

5. Drug Administration

Each drug was dissolved in 0.9% NaCl and was administered through an indwelling 23-gauge butterfly

needle into the ear vein in a volume of 0.5mL/kg. For intracavernosal (IC) drug administration, a 30-gauge needle filled with 4 U/mL heparin solution and connected to PE-10 tubing was inserted into the right corpus cavernosum. IC drugs were administered in a volume of 0.1mL. The change in intracavernosal pressure (ICP) was monitored with each drug dose, and all pressure responses were allowed to return to baseline before the subsequent dose.

6. Data Analysis

Physiological parameters (ICP) was measured at baseline and after PNS with IV or IC administration of zaprinast, sildenafil and vardenafil. Changes in ICP were expressed as a fractional change in corporal pressure in relation to systemic arterial pressure (SAP) (ie, ICP:SAP). Comparisions between the effects of drugs on fractional ICP over time were carried out using analysis of variance and Student's t test.

Results

All 3 agents did not increase in the ICP with IV administration without PNS but showed the increases in the ICP with IV administration following PNS (Group I). Each agents increased the ICP with IC administration without PNS (Group II). Zaprinast enhanced the increase of the ICP in a dose dependent fashion at all doses (30-1,000 μg/kg). With 30 μg/kg and 100 μg/kg doses, zaprinast showed more increase in ICP/SAP in group I than group II ($p<0.05$). With 300 μg/kg and 1000 μg/kg doses, there was no significant difference between group I and group II ($p>0.05$) (Fig. 1). All doses of sildenafil caused dose dependent increases in ICP. With 3 μg/kg and 10 μg/kg doses, the increase in ICP was significantly higher in group II than group II ($p<0.05$). With 30 μg/kg dose, sildenafil showed more increase in ICP/SAP in group II than group I, but there was no significant difference ($p>0.05$) (Fig. 2). With 1 μg/kg dose, vardenafil showed significant increase in ICP/SAP in group II above group I ($p<0.05$). With 3, 10 and 30 μg/kg doses, vardenafil showed the increase in ICP/SAP in group I and II, there was no significant difference between two groups ($p>0.05$) (Fig. 3). The ICP/SAPs with highest doses of zaprinast, sildenafil and vardenafil tested were 0.656, 0.550, 0.654 in group of PNS with intravenous administration and 0.625, 0.609, 0.666 in group of intracavernosal administration without PNS, respectively (Fig. 1, 2, 3).

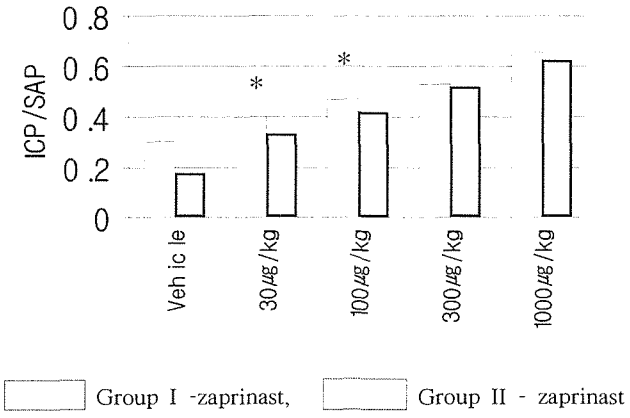

Fig. 1. Comparison of the increase of intracavernosal pressure (ICP)/systolic arterial pressure (SAP) after intravenous (IV) & PNS (pelvic nerve stimulation) (Group I) and intracavernosal (IC) treatment (Group II) with zaprinast (*: $p<0.05$)

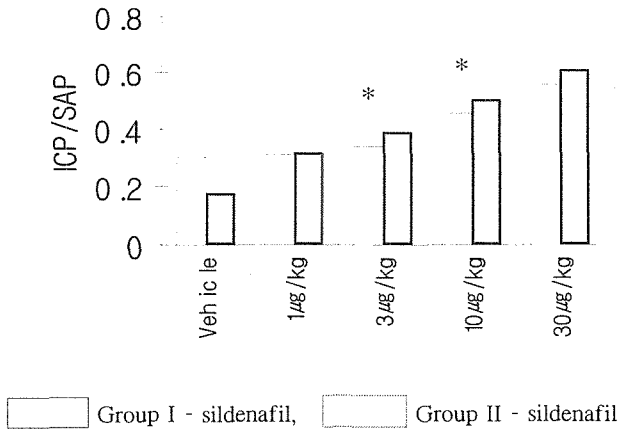

Fig. 2. Comparison of the increase of intracavernosal pressure (ICP)/systolic arterial pressure (SAP) after intravenous (IV) & PNS (pelvic nerve stimulation) (Group I) and intracavernosal (IC) treatment (Group II) with sildenafil (*: $p<0.05$

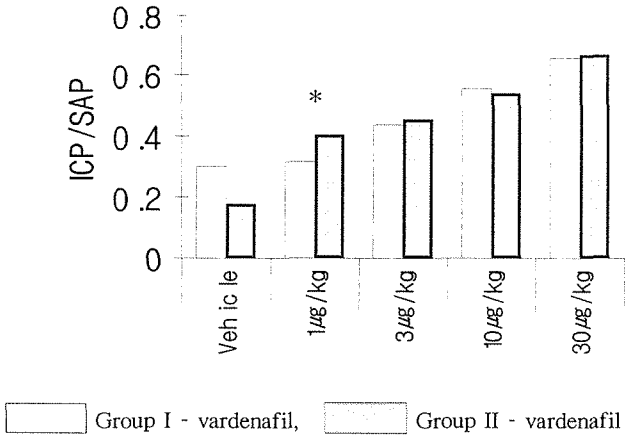

Fig. 3. Comparison of the increase of intracavernosal pressure (ICP)/systolic arterial pressure (SAP) after intravenous (IV) & PNS (pelvic nerve stimulation) (Group I) and intracavernosal (IC) treatment (Group II) with vardenafil (*: $p<0.05$)

Discussion

In response to sexual stimulation, cavernous non-adrenergic, non-cholinergic parasympathetic nerves and endothelial cells release nitric oxide, which stimulate the synthesis of the cytosolic enzyme guanylate cyclase (cGMP) via binding the heme moiety of soluble guanylyl cyclase. This promotes production of cGMP from GTP in cavernosal smooth muscle cells of penis. Cyclic GMP binds to cGMP-dependent protein kinase (protein kinase G) and to cGMP-dependent ion channels. The resulting molecular cascade decreases intracellular calcium levels, allowing relaxation of smooth muscle cells in the cavernosal bodies. Penile blood flow increases and sinusoidal spaces expand, preventing venous outflow of blood and resulting in erection. It is accepted that cAMP pathway also played a role of intracellular secondary messenger that was concerned in penile erection as well as NO/cGMP pathway but the later was more prominent than cAMP pathway in penile erection.[7] PDE terminates cGMP-mediated smooth muscle relaxation via the hydrolysis of phosphodiester bond of cGMP and promotes the restorage of smooth muscle contraction and the consequent penile detumescence. These PDE enzymes are widely distributed in tissues throughout the body, not confined only to penile corpus cavernosum and comprise 13 identified families and their isoforms, which are distinguished by their substrate specificities and tissue concentrations. For decades, non-selective PDE inhibitors, such as caffeine and theophylline, have been used to investigate physiological effects of cyclic nucleotides and PDE activity. But these inhibitors lack potency and specificity because they block catalytic acivity of almost all known PDEs, including PDE5. And it has been established that PDE5 was the predominant isozyme in human corpus cavernosum smooth muscle tissue and responsible for the hydrolysis of cGMP to GMP.[8] So, compounds that potentiate the nitric oxide-cGMP cell-signaling system via inhibition of PDE-catalytic activity have been developed and they revolutionized the management of erectile dysfunction. But it has been generally accepted that these PDE5 inhibitors need a sexual stimulation for the penile erection. To whether these agents produce an erection in the absence of sexual stimulation, Rosen et al[4] reported that these agents did not initiate or produce an erection in the absence of sexual stimulation with consequent nitric oxide release, but Bischoff and Schneider[5] reported that these agents produced an erectile responce with high-dose administration of PDE 5 inhibitors. Nowadays, it is reported that PDE5 inhibitors, in nanomolar serum levels, facilatates penile erection only during sexual stimulation, but intracavernosal PDE5 inhibitors, at tissue levles approaching millimolar concentrations, can cause penile erection in the absence of sexual stimulation.[6] The administered doses of zaprinast, sildenafil and vardenafil in this study were 30-1,000 μg/kg, 1-30 μg/kg and 1-30 μg/kg, respectively. When intravenoulsy administrated, these doses were applicable to 1/500 in penile tissue. All 3 agents did not increase in the ICP with IV administration. But they showed the increases in the ICP with IV administration following PNS and IC administration. Choi et al[9] reported that ICP/SAP with PNS of 2, 4, 8, 16 and 32Hz were 0.39 ± 0.05, 0.69 ± 0.03, 0.82 ± 0.08, 0.95 ± 0.13 and

0.97±0.1 in Male New Zealand white rabbits, respectively. In this study, we stimulated with 2.5Hz to minimize the effect of PNS to penile erection and estimate the effects of administrations of PDE5 inhibitors and increment of dosage to ICP accurately. The balance between the synthetic activities of guanylate cyclase and degradative activities of phosphodiesterase maintains the steady state concentration of cGMP in corpus cavernosum and phosphodiestrase has more affinity to cGMP than guanylate cyslase.[10, 11] Resting levels of cGMP in cavernosal smooth muscle cells remain low (steady state) due to high activity of the type 5 PDE in the absence of sexual stimulation. So, even modest manipulations of the activity of the enzyme through local administration of a PDE inhibitor to the penis could result in meaningful elevations in cGMP above the steady state concentration and subsequently in an erectile response when there is little or no release of NO from non-adrenergic, non-cholinergic nerve fibers by sexual stimulation.[12,13] Also, McAuley et al[6] reported that sildenafil maintained cavernosal smooth muscle relaxtion in spite of administraton of guanylate cyclase inhibitor and NO inhibitor. And they thought that sildenafil may provoke penile erection because it acts as a cGMP analog at high dose medication due to structural similarity with cGMP and activate the substance, such as protein kinase G which controlled by cGMP. Although these hypothesis remain to be further elucidated, it may be that PDE5 inhibitors, in nanomolar serum levels, faciliatates penile erection only during sexual stimulation, but intracavernosal PDE5 inhibitors, at tissue levles approaching millimolar concentrations, can cause penile erection in the absence of sexual stimulation.[8]

We also observed that zaprinast, sidenafil and vardenafil increased ICP in IC administration without PNS in all doses and ICP increased in a dose dependent fashion at response to IV administration with PNS as well as IC administraion without PNS.

With 30 and 100 μg/kg doses, IV administration of zaprinast with PNS significantly increased ICP above the respons to the IC administration. But with 300 and 1000 μg/kg doses, IV injection of zaprinast with PNS increased ICP/SAP, which was not significantly greater than that elicited by the IC injection. So, we confirmed that IC administration of enough dosage without PNS make similar penile erection to IV administration with PNS.

With all doses, IC administration of sildenafil significantly increased ICP above the respons to the IV administration with PNS. With 30 μg/kg doses, IC injection of sildenafil increased ICP/SAP, which was not significantly greater than that elicited by the IV injection with PNS and it is thought that enough erectile response was made by the administration of enough sildenafil. With 1 μg/kg dose, IC administration of vardenafil significantly increased ICP above the respons to the IV administration with PNS. With 3, 10 and 30 μg/kg doses, intracavernous injection of vardenafil caused dose dependent increases in ICP/SAP, which was not significantly greater than that elicited by the IV injection with PNS.

PDE5 is found in relatively high concentration in only a limited number of tisues, such as the corpora cavernosum of the penis, smooth muscle of the systemic and pulmonary vasculature, platelets and visceral smooth muscle[14], thus the most common treatment-related adverse events, such as headache, flushing, indigestion and nasal congestion, are all directly related to PDE5 inhibition and incidences of the adverse events were dose related. Although it is invasive, local IC administration of PDE5 inhibitors may be an alternative intracavernosal injection therapy when oral administration was contraindicated or the established ICI with PGE_1 etc was ineffective.

In this stujdy, sildenafil showed 300 times more potent, comparing with zaprinast in the increase in the ICP. Vadenafil shows 3 times more potent, comparing with sildenafil, and it consistent with the difference of inhibition constant (vardenafil=4.5nM, sildenafil=14.7nM) which Kim et al[14] researches with penile canernosal smooth muscle.

Conclusion

The IV administration of PED5 inhibitors (zaprinast, sildenafil, vardenafil) did not increase ICP at all dosage invested, but PNS after IV administration of PDE5 inhibitors made dose-dependent increases of the ICP. IC administration of PDE5 inhibitors without PNS showed similar effects in the ICP at same dosages. We confirmed that IC administration was more potent and effective route of administration for penile erection than IV.

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