ORIGINAL ARTICLE

Oral phosphodiesterase-5 inhibitors: effect of heme oxygenase inhibition on cGMP signalling in rat cavernous tissue

M. T. Abdel Aziz[1], T. Mostafa[2], H. Atta[1], L. Rashed[1], S. A. Marzouk[1], E. M. Obaia[1], D. Sabry[1], A. A. Hassouna[1], A. M. El-Shehaby[1] & A.T. Abdel Aziz[1]

1 Molecular Biology Unit, Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt;
2 Department of Andrology & Sexology, Faculty of Medicine, Cairo University, Cairo, Egypt

Keywords
cGMP—erectile dysfunction—heme oxygenase—PDE inhibitors — sildenafil—tadalafil—vardenafil

Correspondence
Professor Dr Taymour Mostafa, Department of Andrology & Sexology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt.
Tel.: +20 105 150 297;
Fax: +20 2363 2297;
E-mail: taymour1155@link.net

Accepted: February 14, 2007

Summary

This work postulated that heme oxygenase (HO) is partly responsible for controlling phosphodiesterase-5 inhibitor actions by modulating cyclic guanosine monophosphate (cGMP) cavernous tissue levels. Five hundred and four male Sprague–Dawley rats, divided into five groups, were investigated. Group 1 ($n = 72$) included controls, group 2 ($n = 72$) received sildenafil citrate (Viagra^R) orally, group 3 ($n = 72$) received vardenafil hydrochloride (Levitra^R), group 4 ($n = 72$) received tadalafil (Cialis^R). Group 5 ($n = 216$), subdivided into three subgroups (A, B and C, 72 each), received the same dose of each drug with the HO inhibitor, Zn protoporphyrin. Eight rats from each group/subgroup were killed at 0.5, 1, 2, 3, 4, 6, 18, 24 and 36 h when cGMP levels in the cavernous tissues were estimated. Cavernous tissue cGMP levels increased significantly in sildenafil, vardenafil and tadalafil-treated rats compared to the controls with significant decreases after HO inhibition. It is concluded that the effects of these PDE-5 inhibitors in rat cavernous tissue are partly mediated through HO activity via the cGMP signalling pathway.

Introduction

Penile erection is a neurovascular phenomenon that depends upon neural integrity, a functional vascular system, and healthy cavernosal tissues. Normal erectile function involves three synergistic and simultaneous processes: neurologically mediated increase in penile arterial inflow, relaxation of cavernosal smooth muscle and restriction of venous outflow from the penis (Giuliano *et al.*, 1995; Giuliano & Rampin, 2004).

Nitric oxide (NO) plays a key role in the physiology of penile erection process (Pickard *et al.*, 1995), with the endothelial NO synthase (eNOS) as one of its main sources in the cavernous tissue (Bloch *et al.*, 1998; Burnett, 2002). The most important physiological target of NO in the penis is the haem moiety of soluble guanylate cyclase (Mizusawa *et al.*, 2002). NO diffuses to the adjacent smooth muscle cells stimulating guanylate cyclase converting guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which induces a substantial increase in intracellular cGMP, causing smooth muscle relaxation. Its action is primarily through the cGMP-dependent kinase I, which alters intracellular Ca^{2+} levels. The physiological actions of cGMP are terminated by the hydrolysis of the 3′5′ bond by the type 5 phosphodiesterase (Hedlund *et al.*, 2000; Craven *et al.*, 2004).

Phosphodiesterase-5 (PDE-5) inhibitors selectively act on the erectile tissue causing penile smooth muscle relaxation and vasodilatation leading to penile erection (Schwarz *et al.*, 2006). The Food and Drug Administration (FDA) had approved three PDE-5 inhibitors for treatment of erectile dysfunction: sildenafil citrate (Viagra^R, Pfizer, NY, USA), vardenafil HCl (Levitra^R, Bayer AG, Germany), and tadalafil (Cialis^R, Lilly ICOS LLC, Indianapolis, IN, USA). All three drugs have similar efficacy and mechanism of action. Sildenafil and vardenafil have similar molecular

structure, but tadalafil is structurally different. Sildenafil and vardenafil both have half-lives of about 4 h, whereas the half-life of tadalafil is 17.5 h (Wright, 2006).

Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of haem, converting haem to biliverdin, during which iron is released and carbon monoxide (CO) is emitted (Maines, 2004). CO, a gaseous messenger similar to NO, has been demonstrated to share many properties with NO including activation of guanyl cyclase, signal transduction and gene regulation. Localisation of NOS and HO enzymes in blood vessels and autonomic nervous system strongly implies a possible coordinated physiological role for these two molecules (Wang *et al.*, 2003). CO derived from HO was demonstrated to act as a signalling molecule exhibiting antihypertensive, antiproliferative and antiapoptotic properties (Motterlini *et al.*, 1998; Brouard *et al.*, 2000; Durante, 2003).

The cellular cascade responsible for the effects exerted by CO involves different effectors. Guanylate cyclase is one of the first identified and seems to contribute to the regulation of vascular tone, neurotransmission and cell proliferation (Ingi *et al.*, 1996; Sammut *et al.*, 1998; Nakao *et al.*, 2005). Potassium channels are a second pathway utilized by CO to exert biological functions such as CO-induced vasorelaxation (Liu *et al.*, 1999). In addition, CO appears to act via mitogen-activated protein kinase (MAPK) pathways and decreased c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) phosphorylation (Morse *et al.*, 2003). It is noted that the targets discovered so far for CO-mediated endogenous functions are in common with those used by NO (Ryter *et al.*, 2004). The similarity between CO and NO as gaseous messengers has been previously emphasized (Foresti & Motterlini, 1999); however, as more investigations shed light on the mechanisms underlying the effects of CO, it is becoming evident that the molecular and biochemical pathways characterizing the reactions of the two gases are different and probably reflect their diverse chemical properties and reactivity (Roberto *et al.*, 2005).

Materials and methods

Five hundred and four male Sprague–Dawley rats were used in this study (weight 150–200 gm), bred in accordance with the ethical standards of institutional committee guidelines (IRB) and Institutional Animal Care and Use Committee (IACUC). All animals were housed under constant environmental conditions and were fed on standard laboratory rat diet and distilled water. Acclimatisation was achieved in 7 days, after which the proposed doses of tested materials were delivered orally through gastric tube, at the fixed time 10:00–12:00 AM. They were divided into five groups: control group 1 ($n = 72$) rats received regular diet, group 2 ($n = 72$) rats received sildenafil citrate (8 mg kg^{-1} body weight, equivalent to 100 mg dose in 70 kg adult man according to Paget's table of experimental studies) (Paget & Barnes, 1964), group 3 ($n = 72$) rats received vardenafil hydrochloride (1.6 mg kg^{-1} body weight, eq. 20 mg dose in adults), group 4 ($n = 72$) rats received tadalafil (1.6 mg kg^{-1} body weight, eq. 20 mg dose in adults). Group 5 ($n = 216$) rats, subdivided into three subgroups (A, B and C, 72 rats each), received the same dose of each drug with an HO inhibitor (Zn protoporphyrin, 50 μg kg^{-1}).

Eight rats from each set were killed by cervical dislocation after 0.5, 1, 2, 3, 4, 6, 18, 24 and 36 h successively. Cavernous tissues were dissected, kept in 0.1 N HCl to inhibit phosphodiesterase enzyme. Stored cavernous tissue samples were homogenised and centrifuged at 6000 ***g*** at 4 °C for 10 min. The supernatant was used for cGMP assay by ELISA kit (R&D Systems, Minneapolis, MN, USA) (Mingone *et al.*, 2003).

Statistical analysis

Numerical data were expressed as mean ± SD. Comparisons were performed by Student's *t*-test and $P < 0.05$ was considered statistically significant.

Results

Cavernous tissue cGMP levels (pmol mg^{-1}) were significantly higher in sildenafil, vardenafil and tadalafil-treated groups compared to the controls (Tables 1–3). Generally, marked increase was evident after 0.5 h, reaching a peak after 1 h, followed by a gradual drop after 3 h. In the sildenafil and vardenafil-treated groups, cGMP returned to the control level after 6 h, while those treated with tadalafil showed slow decrease but still higher than the controls till 36 h (Fig. 1). Rats received an HO inhibitor in addition to the dose of the drugs and demonstrated a significant decrease in cGMP levels compared to those that received the drugs alone. cGMP levels in those that received sildenafil and vardenafil in addition to the HO inhibitor were higher than in the controls in the first 6 h, then decreased below the control level afterwards. cGMP levels in those that received tadalafil and the HO inhibitor were higher than in the controls in the first 18 h, then decreased below it afterwards (Fig. 2).

Discussion

Since the introduction of the PDE-5 inhibitor sildenafil citrate in 1998, there has been a fundamental change in the treatment of ED. The development of two new PDE-5 inhibitors, vardenafil and tadalafil, has added to this

Table 1 Cavernous tissue cGMP levels (pmol mg^{-1}) in sildenafil-treated groups

	0.5 h	1 h	2 h	3 h	4 h	6 h	18 h	24 h	36 h
Group 1 (control)									
Mean	1.06	0.95	2.12	1.04	0.89	1.56	0.67	0.94	0.72
SD	0.22	0.18	0.34	0.24	0.13	0.20	0.11	0.20	0.20
Group 2 (sildenafil)									
Mean	3.77[a]	9.32[a]	6.25[a]	5.24[a]	3.15[a]	0.85	0.84	0.85	0.79
SD	0.43	0.89	0.39	0.45	0.67	0.12	0.15	0.18	0.18
Group 5A (sildenafil + Zn protoporphyrin)									
Mean	2.10[b]	3.60[b]	2.70[b]	1.10[b]	0.90[b]	0.84	0.84	0.84	0.78
SD	0.3	0.4	0.21	0.17	0.18	0.04	0.05	0.12	0.13

[a]Significant difference compared to the controls.
[b]Significant difference compared to group 2.

Table 2 Cavernous tissue cGMP levels (pmol mg^{-1}) in vardenafil-treated groups

	0.5 h	1 h	2 h	3 h	4 h	6 h	18 h	24 h	36 h
Group 1 (control)									
Mean	1.06	0.95	2.12	1.04	0.89	1.56	0.67	0.94	0.72
SD	0.22	0.18	0.34	0.24	0.13	0.20	0.11	0.20	0.20
Group 3 (vardenafil)									
Mean	2.32[a]	5.68[a]	3.84[a]	2.65[a]	2.01[a]	0.79	0.82	0.80	0.82
SD	0.33	0.87	0.42	0.35	0.22	0.19	0.16	0.18	0.17
Group 5B (vardenafil + Zn protoporphyrin)									
Mean	1.90[b]	2.50[b]	1.30[b]	1.00[b]	0.80[b]	0.72	0.61	0.63	0.50
SD	0.2	0.3	0.15	0.13	0.12	0.1	0.1	0.18	0.11

[a]Significant difference compared to the controls.
[b]Significant difference compared to group 3.

Table 3 Cavernous tissue cGMP levels (pmol mg^{-1}) in tadalafil-treated groups

	0.5 h	1 h	2 h	3 h	4 h	6 h	18 h	24 h	36 h
Group 1 (control)									
Mean	1.06	0.95	2.12	1.04	0.89	1.56	0.67	0.94	0.72
SD	0.22	0.18	0.34	0.24	0.13	0.20	0.11	0.20	0.20
Group 4 (tadalafil)									
Mean	2.91[a]	7.80[a]	5.58[a]	4.07[a]	2.37[a]	2.49[a]	2.43[a]	1.72	0.94
SD	0.31	0.77	0.55	0.63	0.38	0.53	0.66	0.46	0.12
Group 5C (tadalafil + Zn protoporphyrin)									
Mean	2.4[b]	2.90[b]	2.10[b]	1.40[b]	1.50[b]	1.20[b]	0.80[b]	0.80	0.78
SD	0.2	0.25	0.2	0.17	0.12	0.14	0.1	0.1	0.1

[a]Significant difference compared to the controls.
[b]Significant difference compared to group 4.

option. This has stimulated academic, clinical and industrial researchers to conduct experiments aimed at understanding the mechanism(s) underlying the erectile process, hoping to develop better treatment modalities (Chen *et al.*, 2004).

In the present study, cavernous tissue cGMP levels were significantly higher in oral PDE-5 inhibitor-treated groups compared to the controls, showing an increase after 0.5 h with a peak after 1 h, followed by a gradual decline after 3 h. Groups treated with sildenafil and vardenafil reached the control levels after 6 h, while the group treated with tadalafil reached the control levels after 36 h. This goes with the half-life of each drug (half-life of sildenafil and vardenafil is 4 h, whereas the half-life of tadalafil is 17.5 h) (Wright, 2006). Porst & Lunglmayr (2005) showed that vardenafil was effective at the 10 mg dose, when the patients utilised its effect within a time window ranging from <0.5 to 12 h. Blount *et al.* (2004) demonstrated that the absolute potency values were similar for each inhibitor, and the relative potencies were vardena-

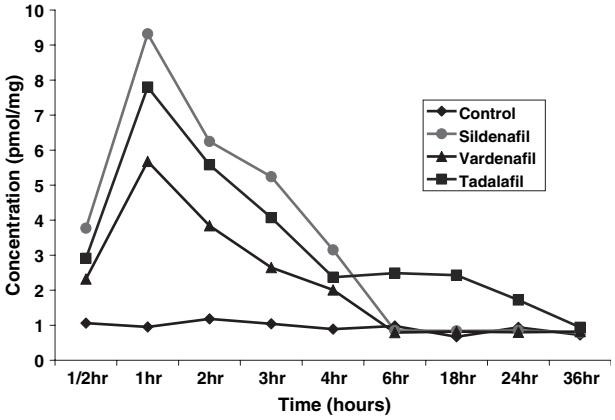

Fig. 1 Cavernous tissue cGMP levels (pmol/mg) in studied groups treated with different oral PDE-5 inhibitors.

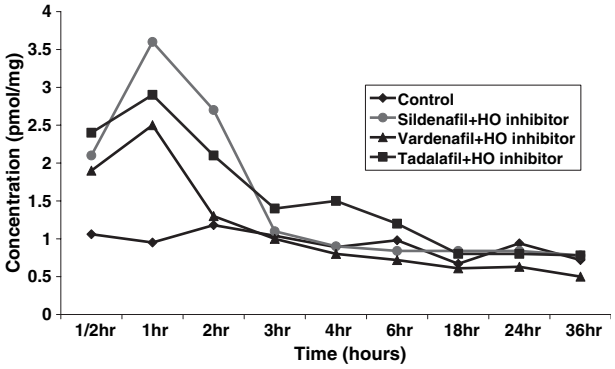

Fig. 2 Cavernous tissue cGMP levels (pmol/mg) in studied groups treated with different oral PDE-5 inhibitors and HO inhibitor.

fil >> tadalafil > sildenafil. Studies of sildenafil and vardenafil analogues demonstrated that higher potency of vardenafil is caused by differences in its double ring.

Rats that received these drugs in addition to the HO inhibitor demonstrated a significant decline in cGMP levels compared to those treated with PDE-5 inhibitors alone. cGMP levels in the subgroups treated with sildenafil or vardenafil in addition to the HO inhibitor were higher than in the controls in the first 6 h, then decreased below them afterwards. In addition, the subgroup treated with tadalafil and HO inhibitor demonstrated cGMP levels higher than the controls in the first 18 h, then decreased below the controls afterwards. The increase in cGMP levels compared to the controls could be explained by cGMP released due to basal NO signalling pathway, while the subsequent decrease can be related to the pharmacokinetics of each drug (i.e. the half-life of each drug).

Like NO, CO binds to the haem moiety of soluble guanylate cyclase (sGC) leading to its activation as well as an increase in cGMP levels (Rich *et al.*, 1994). The ability of CO to activate sGC and increase cGMP is believed to be part of the mechanism that underlies CO vasodilator activity (Wang *et al.*, 1997, 2003). Abdel Aziz *et al.* (2005) indicated that induction of either NOS or HO was equally effective in enhancing the erectile process via upregulating gene expression of the two signalling molecules, NOS and HO, through upregulation of the local tissue levels of cGMP. The role of CO as an NO-like signalling molecule was also supported from studies on HO and NOS knockouts. Furthermore, both HO-1 and HO-2-derived CO have a positive and negative effect on sGC and cGMP levels in vascular endothelial cells (Abraham *et al.*, 1985). Ryter *et al.* (2004) stated that HO protects NO through scavenging of reactive oxygen species (ROS). Thus, HO prevents NO from reacting with ROS, preventing the formation of peroxynitrite and its subsequent degradation, confirming that CO tissue levels parallel NO levels.

It could be concluded that the erectogenic action of PDE-5 inhibitors in the cavernous tissue is partly mediated through HO activity via the cGMP signalling pathway.

Conflict of interest

None.

References

Abdel Aziz MT, El-Asmar MF, Mostafa T, Atta H, Abdel Aziz M, Fouad H, Roshdy N, Rashed L, Sabry D (2005) Effects of nitric oxide synthase and heme oxygenase inducers and inhibitors on molecular signaling of erectile function. *Clin Biochem Nutr (Japan)* 37:103–111.

Abraham NG, Lutton JD, Levere RD (1985) Heme metabolism and erythropoiesis in abnormal iron states. Role of amino-levulinic acid synthase and heme oxygenase. *Exp Haematol* 13:833–843.

Bloch W, Klotz T, Sedlaczek P, Zumbe J, Engelmann U, Addicks K (1998) Evidence for the involvement of endothelial nitric oxide synthase from smooth muscle cells in the erectile function of the human corpus cavernosum. *Urol Res* 26:129–135.

Blount MA, Beasley A, Zoraghi R, Sekhar KR, Bessay EP, Francis SH, Corbin JD (2004) Binding of tritiated sildenafil, tadalafil, or vardenafil to the phosphodiesterase-5 catalytic site displays potency, specificity, heterogeneity, and cGMP stimulation. *Mol Pharmacol* 66:144–152.

Brouard S, Otterbein LE, Anrarther J (2000) Carbon monoxide generated by heme oxygenase-1 suppresses endothelial cell apoptosis. *J Exp Med* 192:1015–1026.

Burnett AL (2002) Nitric oxide regulation of penile erection: biology and therapeutic implications. *J Androl* 23:S20–S26.

Chen Q, Wang N, Yao F, Lu X (2004) Comparison of efficacy and safety of phosphodiesterase type 5 inhibitors in the

treatment of erectile dysfunction. *Zhonghua Nan Ke Xue* 10:302–304.

Craven M, Sergeant GP, Hollywood MA, McHale NG, Thornbury KD (2004) Modulation of spontaneous Ca^{2+} activated Cl-currents in the rabbit corpus cavernosum by the nitric oxide cGMP pathway. *J Physiol* 556:495–506.

Durante W (2003) Heme oxygenase-1 in growth control and its clinical application to vascular disease. *J Cell Physiol* 195:373–382.

Foresti R, Motterlini R (1999) The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. *Free Radic Res* 31:459–475.

Giuliano F, Rampin O (2004) Neural control of erection. *Physiol Behav* 83:189–201.

Giuliano FA, Rampin O, Benoit G, Jardin A (1995) Neural control of penile erection. *Urol Clin North Am* 22:747–766.

Hedlund P, Aszodi A, Pfeifer A, Alm P, Hofmann F, Ahmad M, Fassler R, Andersson KE (2000) Erectile dysfunction in cyclic GMP-dependent kinase I-deficient mice. *Proc Natl Acad Sci U S A* 97:2349–2354.

Ingi T, Cheng J, Ronnett GV (1996) Carbon monoxide: an endogenous modulator of the nitric oxide-cGMP signaling system. *Neuron* 16:835–842.

Liu HJ, Mount DB, Nasjletti A, Wang WH (1999) Carbon monoxide stimulates the apical 70-pS K+ channel of the rat thick ascending limb. *J Clin Invest* 103:963–970.

Maines MD (2004) The heme oxygenase system: past, present, and future. *Antioxid Redox Signal* 6:797–801.

Mingone CJ, Gupte SA, Quan S, Abraham NG, Wolin MS (2003) Influence of heme and heme oxygenase-1 transfection of pulmonary microvascular endothelium on oxidant generation and cGMP. *Exp Biol Med* 228:535–539.

Mizusawa H, Hedlund P, Brioni JD, Sullivan JP, Andersson KE (2002) Nitric oxide independent activation of guanylate cyclase by YC-1 causes erectile responses in the rat. *J Urol* 167:2276–2281.

Morse D, Pischke SE, Zhou Z (2003) Suppression of inflammatory cytokine production by carbon monoxide involves the JNK pathway and AP-1. *J Biol Chem* 278:36993–36998.

Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, Winslow RM (1998) HO-1 derived CO contributes to the suppression of acute hypertensive responses *in vivo*. *Circ Res* 83:568–577.

Nakao A, Neto JS, Kannos S (2005) Protection against ischemia/reperfusion injury in cardiac and renal transplantation with carbon monoxide, biliverdin and both. *Am J Transplant* 5:282–291.

Paget GE, Barnes GM (1964) Evaluation of Drug Activities, Vol. 1. Academic Press, London.

Pickard RS, Powell PH, Zar MA (1995) Nitric oxide and cyclic GMP formation following relaxant nerve stimulation in isolated human corpus cavernosum. *Br J Urol* 75:516–522.

Porst H, Lunglmayr G (2005) Efficacy and tolerability of vardenafil within the time window of 6 h after administration and beyond. Results of a clinical study carried out in 233 urological offices. *MMW Fortschr Med* 147 (Suppl. 1): 27–32.

Rich A, Farrugia G, Rae JL (1994) Carbon monoxide stimulates a potassium selective current in rabbit corneal epithelial cells. *Am J Physiol* 267 (1 Pt 1): C435–C442.

Roberto M, Brian EM, Roberta F (2005) Therapeutic applications of carbon monoxide-releasing molecules. *Expert Opin Invest Drugs* 14:1305–1318.

Ryter SW, Morse D, Choi AM (2004) Carbon monoxide: to boldly go where NO has gone before. *Sci STKE* 20:RE6.

Sammut IA, Foresti R, Clark JE, Exon DJ, Vesely MJJ, Sarathchandra P, Green CJ (1998) Carbon monoxide is the major contributor to the regulation of vascular tone in aortas expressing high levels of heme oxygenase-1. *Br J Pharmacol* 125:1437–1444.

Schwarz ER, Kapur V, Rodriquez J, Rastogi S, Rosanio S (2006) The effects of phosphodiesterase-5 inhibitor use on different organ systems. *Int J Impot Res* 21:135–140.

Wang R, Wang Z, Wu L (1997) Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol* 121:927–934.

Wang J, Lu S, Moenne P, Montellano OR (2003) Interaction of nitric oxide with human heme oxygenase-1. *J Biol Chem* 278:2341–2347.

Wright PJ (2006) Comparison of phosphodiesterase type 5 (PDE5) inhibitors. *Int J Clin Pract* 60:967–975.

