Short Communication

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Screening of new compounds for the management of erectile dysfunction

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ABSTRACT

Objective/Background: Erectile dysfunction, a male sexual dysfunction generally develops due to impairment of relaxation of penile smooth muscles and arteries. Inhibitors of phosphodiesterase type 5 (PDE5) enzyme like in sildenafil, vardenafil, tadalafil etc. are in clinical use for the management of erectile dysfunction. Ongoing research focuses on development of potent medicine with fewer side effects. Objective of this article is to discuss and demonstrate procedures available to screen new compounds for the management of erectile dysfunction. **Methodology:** Many animal models (mice, rat, cat, rabbit, dog etc.) are available to screen new chemical compounds for increasing erectile function. *In vitro* (using isolated corpus cavernosum smooth muscle of penile tissue) and *in vivo* (measuring intracavernosal pressure) studies may be performed for screening new compounds. In this article, we discuss about two such screening procedures using rat as an animal model. **Results and discussion:** Compounds those relax isolated rat corpus cavernosum smooth muscles significantly in vitro may increase penile erection in humans. Similarly, the compounds those increase intracavernosal pressure by relaxing erectile smooth muscle and penile arteries in rat may increase erectile function in humans. **Conclusion:** Rat is a suitable model for screening new compounds to manage erectile dysfunction.

Key words: Corpus cavernosum smooth muscle, intracavernosal pressure, mean arterial pressure

INTRODUCTION

Erectile dysfunction (ED) is a male sexual disorder that mainly affects aged males, smokers and persons with conditions such as diabetes, blood pressure, and depression. It is defined as the inability of men to attain and maintain penile erection required for satisfactory sexual intercourse.

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Sexual function is regulated by central nervous system, and it is also controlled peripherally. Penile erection is the end result of relaxation of penile smooth muscles and arterioles, therefore, inability of penile smooth muscles to relax in the presence of sexual stimuli may lead to ED.[1,2]

Penis contains three smooth muscle structures i.e., one corpus spongiosum that surrounds urethra and two corpora cavernosa (singular-corpus cavernosum) whose relaxation is responsible for penile erection. New compound that relaxes isolated corpus cavernosum smooth muscles (CCSMs) significantly *in vitro* would be helpful therapeutic candidate in the management of ED. Italiano *et al.*, suggested using corpus cavernosum of rat for investigating erectile pharmacology as penile structure

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of men and rat are similar.[1,3] Quinlan *et al.* showed that rat can be used as a model for the study of penile erection. Major pelvic ganglions (MPGs) lie on either side of the dorsolateral lobes of the prostate and autonomic nerves (from MPGs) those that innervate penis are known as cavernous nerves (CN). Stimulation of CN or MPG can increase intracavernous pressure (ICP), a measure of penile erection.[4,5] New compound that increases ICP significantly would be helpful in the management of ED. The new compound that relaxes CCSMs may decrease blood pressure by relaxing blood vessels. Therefore, mean arterial pressure (MAP) is also measured along with ICP.

MATERIALS AND METHODS

Effect of new compound on isolated rat corpus cavernosum

Anesthetize male Wistar rat weighing 150-250 g with ketamine (60 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) (any other anesthesia can be used). Deglove the prepuce foreskin covering penis followed by cutting dorsal penile nerves and dorsal penile vein from the dorsal surface, and corpus spongiosum covering urethra from the ventral surface. Microsurgically remove tunica albuginea that surrounds corpus cavernosum and cut along the tunica albuginea that holds two corpus cavernosa. Cut both the ends of shaft (body) of penis to get two corpus cavernosa of size 3 mm × 3 mm × 15 mm. Wash the tissues with Krebs-Henseleit (KH) physiological salt solution of following composition (in mM): 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.5 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, maintained at 37°C and aerated with carbogen gas (95% O_2 and 5% CO_2). Tie one end of the tissue to steel hook at the bottom of organ chamber containing KH solution maintained at required temperature, aerated with carbogen gas and another end to force transducer (Model no: MLT0201; ADInstruments, Australia connected to PowerLab/8SP data acquisition system, Chart software, version 7.0; ADInstruments, Australia) using cotton/silk thread. Exert 500 mg of tension to the tissue and equilibrate for 1 h, replacing KH solution every 1 h. Contract the tissue with 3 μM phenylephrine (sub-maximal dose) and once the tissue attains maximum contraction, record the relaxant effect of either vehicle, new compound or sildenafil, the standard drug used in the management of ED (Figures 1 and 2).

Measurement of ICP and MAP

Anesthetize the male rat as described earlier and expose the penile tissue and deglove the prepuce. Locate crus (basal portion of corpus cavernosum) and place the 24G needle connected to PE tubing (filled with 200 IU heparin saline) connected to the pressure transducer that in turn should be connected to AD Instrument data acquisition system (or any other data acquisition system). Push 10-20 μL of heparin saline solution into the crus and observe the deflection in penis that confirms that needle is in the crus. Remove two testes from the scrotal sac and out of the peritoneal cavity. Lift the seminal vesicle (SV) and place CN/MPG on the dorsolateral lobe of prostrate at the base of SV on the bipolar steel electrode (wire diameter 1 mm or less; distance between two poles: 3 mm) (Figure 3).

Shave the hair of the neck and make a small cut on the skin of the neck. Locate the carotid artery supplying to brain on either side of the trachea along with the vagus nerve. Detach the vagus nerve from carotid artery taking utmost care not to damage/cut vagus nerve. Cannulate carotid artery for placing Millars mikrotip catheter (SPR-320, pressure transducer marketed by AD Instrument, Australia; use 3F size for rat, 2F size for mice; PE tubes connected to any other pressure transducer also can be used)[6] (Figure 4).

Stimulate CN/MPG with 5 ms pulses of 5V at a frequency of 12 Hz for 30 s and record the change in ICP and MAP in rats treated with vehicle, new compound or sildenafil (Figure 5). Voltage, frequency and duration of stimulation can be optimized for your own experiment.

Note: After, needle is inserted to crus of corpus cavernosum, ICP increases up to 10-20 mmHg. After a

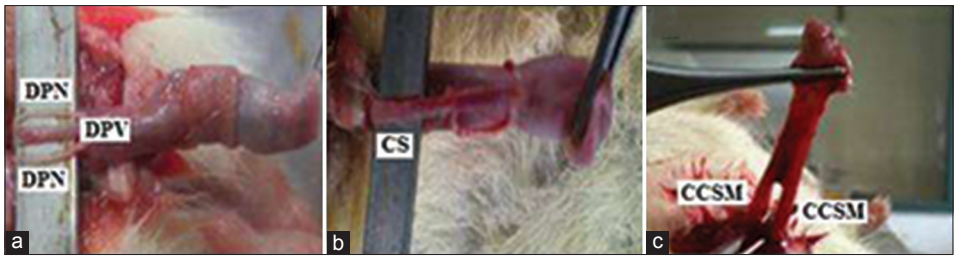

Figure 1: Anatomy of rat penile tissue and isolation of corpus cavernosum smooth muscle (CCSM). Deglove prepuce of the rat penile tissue (a) Locate and cut two dorsal penile nerves and one dorsal penile vein (b) Remove corpus spongiosum covering urethra (c) Locate two CCSMs on shaft (body) of the penis and separate by cutting across the tunica albuginea that covers both CCSM

stable baseline of ICP is maintained, CN/MPG can be stimulated to see the effect of treatment on ICP. After the stimulation ceases, ICP return to normal. The same procedure can be repeated 3 times for an animal in the interval of 10 min each for checking reproducibility of the data collected. Maximum ICP recorded area under the curve, and ICP/MAP can be used for statistical calculation.

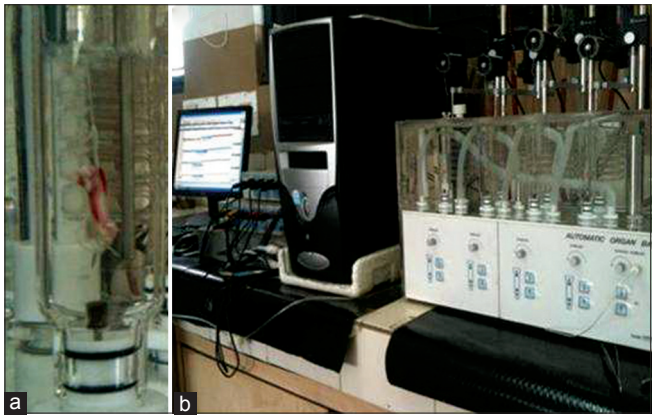

Figure 2: Screening of new compound using isolated corpus cavernosum smooth muscle (CCSM) *in vitro*. (a) CCSM is mounted in organ bath (b) Recording the effect of vehicle, new compound and sildenafil using 4 channel organ bath and AD Instrument data acquisition system

RESULTS

Relaxation of isolated corpus cavernosum smooth muscle and increase in intracavernosal pressure provide information about erectile function.

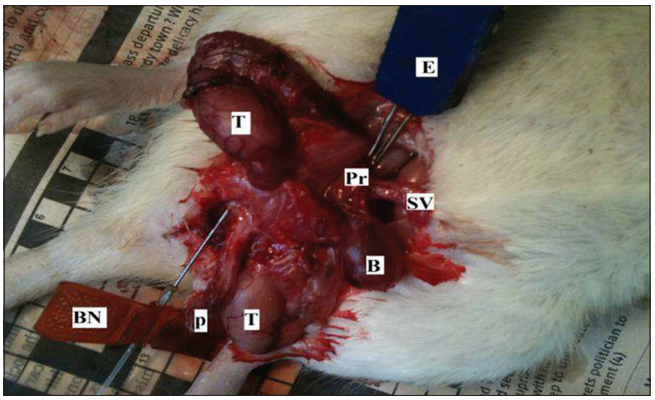

Figure 3: Measurement of (intracavernous pressure) BN: Butterfly needle, P: Penis, T: Testis, B: Bladder, Pr: Prostrate, SV: Seminal vesicle, E: Electrode

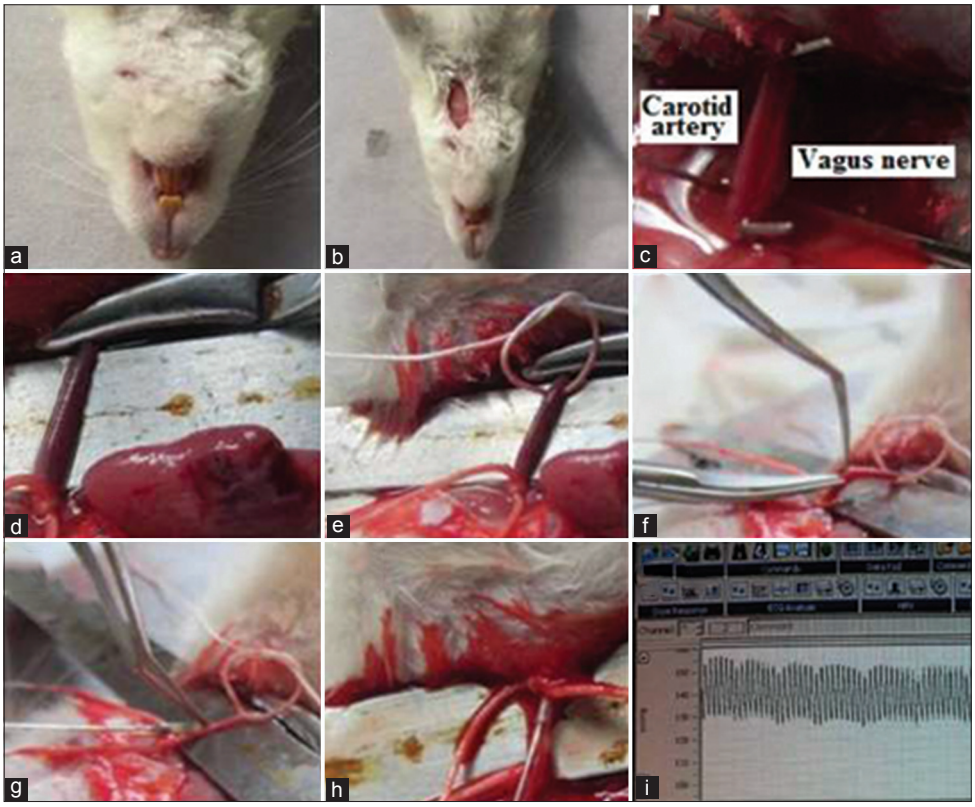

Figure 4: Measurement of mean arterial pressure (MAP). (a) Anesthetize rat and was and shave the hair on the neck (b) Make an incision into the skin of neck and separate the muscles (c) Locate the carotid artery along with vagus nerve and separate the artery (d) Tie the carotid artery with thread (silk thread, 3-0 size is better) and block it by bulldog clip, 1.5 cm apart (e) Tie another thread loosely (f) Lift the carotid artery by an artery forceps and cut it slightly with an iris scissor (g) Open partially collapsed artery by curved iris forceps and insert Millars mikro-tip pressure catheter (3F size) in to the artery (h) Secure the inserted catheter (with thread tied [around the catheter] over artery) and remove bulldog clip (i) measure MAP using AD Instrument data acquisition system

Table 1: Optimization of experiment

Nature of trouble	Action to be taken
Isolated tissue is not responding to pharmacologic treatment	Partially remove tunica albuginea
	Prepare fresh solution of pharmacological agents
	If KH solution is getting precipitated, prepare fresh solution
	Mount new tissues
ICP does not increase after nerve stimulation	Check if PE tubing is blocked by clotting of blood in it. If it is due to clotting of blood then remove needle (with PE tubing) from crus and push approximately 10-20 µL of heparine saline solution to remove the clot and again put the needle in crus
	Check whether nerve/MPG is on electrode or not
	Check whether you have selected suitable stimulating parameter or not
	Check whether stimulator and electrodes are working or not
	Check whether tip of the needle is in crus or not
	Check integrity of CN/MPG
	Check the concentration of heparine in solution and use the solution within 6-12 h
CN is getting burnt	Use an electrode of less diameter because more is the diameter more will the amount of heat generated
ICP data is not reproducible after subsequent stimulation	Wait for about 10 min before stimulating nerve/MPG again
Animal is waking up during ICP and MAP measurement	Use supplemental dose of anesthesia to increase the duration of anesthesia

ICP: Intracavernous pressure, MAP: Mean arterial pressure, CN: Cavernous nerves, MPG: Major pelvic ganglion, KH: Krebs-Henseleit

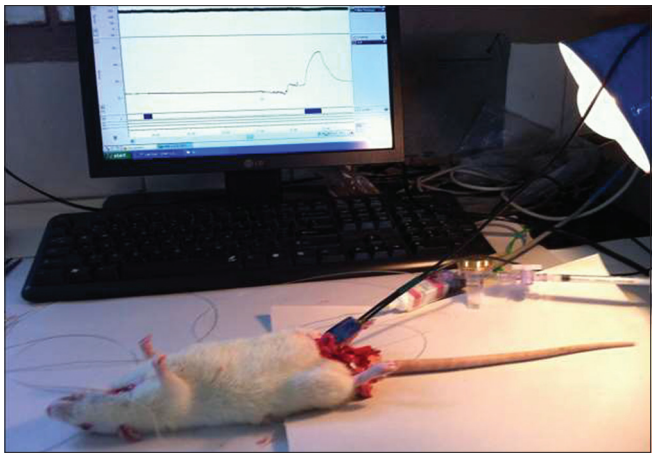

Figure 5: Measurement of intracavernous pressure (ICP) and mean arterial pressure (MAP). First, second and third channels on computer screen are displaying MAP (deep green line), ICP (pink line) and stimulation (blue line) respectively

DISCUSSION

Measurement of ICP and MAP is a gold standard for determining the effect of new compound on erectile function. Sexual behavior study is an alternative method for screening new compound for their effect on sexual function of male rats in the presence of female rats. However, the study is more time taking as we have to train animals before the actual study and require female rats also. Female rats need to be administered estrogen (or derivative) and progesterone to bring them to estrous (heat) phase so that they allow male rats to copulate with them. To avoid pregnancy of female rats they may be ovariectomised that need 6-10 days for the wound to heal.[7] The study also does not provide any data on the effect of treatment on cardiovascular function. The behavior of animals also may be affected by presence of study personnel, so it is suggested to use video camera for recording sexual behavior and then analyze individual parameters of sexual behavior afterwards. The positive point of sexual behavior study is that the effect of treatment can be studied in reproduction. For past decade or so, most of the researchers have relied more on the ICP and MAP studies than the sexual behavior studies simply because the collected data are accurate, takes less time to acquire the data (Table 1).

CONCLUSION

Rat is a suitable model to screen new compounds for the management of erectile dysfunction.

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REFERENCES

1. Andersson KE. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. Pharmacol Rev. 2011;63:811-59.
2. Guay AT, Spark RF, Bansal S, Cunningham GR, Goodman NF, Nankin HR, *et al.* American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of male sexual dysfunction: A couple's problem--2003 update. Endocr Pract. 2003;9:77-95.
3. Italiano G, Calabrò A, Pagano F. A simplified *in vitro* preparation of the corpus cavernosum as a tool for investigating erectile pharmacology in the rat. Pharmacol Res. 1994;30:325-34.

4. Quinlan DM, Nelson RJ, Partin AW, Mostwin JL, Walsh PC. The rat as a model for the study of penile erection. J Urol. 1989;141:656-61.
5. Tocharus C, Smitasiri Y, Jeenapongsa R. Butea superba Roxb. enhances penile erection in rats. Phytother Res. 2006;20:484-9.
6. Rajasekaran M, White S, Baquir A, Wilkes N. Rho-kinase inhibition improves erectile function in aging male Brown-Norway rats. J Androl. 2005; 26:182-8.
7. Agmo A. Male rat sexual behavior. Brain Res Brain Res Protoc. 1997;1:203-9.