The Penile Erection Efficacy of a New Phosphodiesterase Type 5 Inhibitor, Mirodenafil (SK3530), in Rabbits with Acute Spinal Cord Injury

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ABSTRACT. Mirodenafil (SK3530) is a new potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5). Recent clinical trials have demonstrated that mirodenafil is an effective treatment for erectile dysfunction. Its mechanism of action is enhancement of nitric oxide (NO) induced cGMP formation resulting in significant relaxation of the corpus cavernosum (CC). The aim of this study was to investigate the oral efficacy of mirodenafil in an acute spinal cord-injured rabbit model. Mirodenafil or sildenafil citrate was given orally to male rabbits with a surgical transection of the spinal cord at the L2-L4 lumbar vertebra or ischemic-reperfusion spinal cord injury (SCI). Erections were evaluated in a time-course manner by measuring the length of the uncovered penile mucosa. In the transection SCI model, penile erections were induced at 0.3, 1 and 3 mg/kg of mirodenafil but sildenafil only showed an erectile response at 3 mg/kg. The effects of 1 and 3 mg/kg of mirodenafil were significantly increased by intravenous injection of sodium nitroprusside (SNP), a nitric oxide donor. In the ischemic-reperfusion injury model, 3 mg/kg of either mirodenafil or sildenafil produced a penile erection response. After injection of SNP, the lengths of immediate penile erections were significantly increased in the 1 and 3 mg/kg mirodenafil and 3 mg/kg sildenafil groups. The onset of erectile activity was faster with mirodenafil than with sildenafil citrate. These results demonstrate that mirodenafil may be useful for treating erectile dysfunction in patients with a spinal cord injury.

KEY WORDS: mirodenafil, penile erection, phosphodiesterase inhibitor, SK3530, spinal cord injury.

The advent of phosphodiesterase type 5 (PDE5) inhibitors as oral therapy for erectile dysfunction (ED) ushered in a revolution in clinical management of this condition. Oral ED medications influence the complex neurological, vascular and humoral process underlying penile erection. PDE5 inhibitors influence local regulatory mechanisms of erection, potentiating the smooth muscle relaxing effects of nitric oxide (NO) on resistance arteries and trabecular smooth muscle within the corpus cavernosum [4].

Across the world, approximately 152 million men have ED, including about 31 million men in Europe and 18 million men in the US [6, 24]. With anticipated increases in the median ages of Western industrialized societies and population growth in developing nations, the number of cases is projected to increase by about 170 million worldwide over the next 25 years. A wide variety of treatment options are now available for patients with ED, ranging from hormone replacement, counseling, non-invasive devices and oral therapies to injectable agents, penile implants and vascular surgery. As a result, almost all men with ED of any type can receive effective treatment. The process of care model designates counseling, vacuum pump, and PDE 5 inhibitors such as sildenafil as first-line options for ED in the primary care setting [12].

From the neurological perspective, penile erection is caused by a change in activity of efferent autonomic pathways to erectile tissues and somatic pathways to perineal striated muscles [3]. The spinal cord contains the cell bodies of autonomic and somatic motoneurons that innervate peripheral targets. Sympathetic outflow is mainly anti-erectile, sacral parasympathetic outflow is pro-erectile and pudendal outflow, through contraction of perineal striated muscles, enhances an already present erection [13, 18]. The parts of the spinal network that control erection consist of neurons in the spinal midbrain and forebrain nuclei after mating, a condition that recruits and sensory genital afference [11]. In all mammals studied so far, the main pro-erectile peripheral pathway is represented by the pelvic and cavernous nerve. Electrical stimulation applied on the sacral root or pelvic and cavernous nerves in conscious and anesthetized animals elicits, depending on the recordings performed, visible penile erection, increased penile volume, engorgement of the penis with blood, increased blood leakage from the sectioned penis and increased intracavernous pressure [31].
Spinal cord injury (SCI) results in injury to both neural and vascular elements. The extent to which the vascular injury contributes to secondary pathogenesis is dependent on not only the initial disruption of blood vessels, leading to prominent intraparenchymal hemorrhage, but also progressive disruption of the blood-spinal cord barrier coincident with infiltration of inflammatory cells. These events influence both acutely and chronically injured spinal cords and define, in part, the extent of functional recovery. Erectile dysfunction is a common complication in spinal cord injury patients. Experimental models of spinal cord injury include contusions, compression, ischemia and crush injuries. Each model generates injuries that mimic certain clinical aspects of mechanical damage and the ensuing posttraumatic ischemia. Researchers have also used either transection or hemisection to study injury. Transection and hemisection are less clinically relevant but offer the distinct advantage of consistent reproducibility. The most sensitive organ is the spinal cord, and ischemia can produce paraparesis or paraplegia after operation on the thoracic aorta. In this experiment, spinal cord injury was induced by the ischemic-reperfusion and transection method.

SK Chemicals has recently developed a PDE5 inhibitor, mirodenafil (SK3530), for use as a potential oral drug for treatment of ED. Mirodenafil, 5-ethyl-2-{5-[4-(2-hydroxy-ethyl)-piperazine-1-sulfonyl]-2-propoxy-phenyl}-7-propyl-3,5-dihydro-4\(\text{H}\)-pyrrolo[3,2-\(d\)]pyrimidin-4-one (Fig. 1), is a new potent and selective inhibitor of cGMP-specific PDE5 that has \(pK_a\) value of 5.99 and \(\log P\) partition coefficient (octanol/water) of 3.67 [20]. It differs from sildenafil in the \(N-\) or \(O-\)side chain and has a dihydropyrrrole ring instead of a pyrazole ring. These modifications confer a more potent and selective inhibitory effect on PDE5 to mirodenafil than those of sildenafil [26]. Pharmacokinetic data for mirodenafil suggests that it is relatively well absorbed in the gastrointestinal tract and shows linear pharmacokinetics over the investigated dose range [32]. Based on unpublished data, mirodenafil for which is currently under phase III clinical investigation, appears to be safe and effective for treatment of male ED [25, 26, 32].

The aim of this study was to assess the effect of a selective PDE5 inhibitor, mirodenafil (SK3530), in rabbits with acute spinal cord injury after oral administration and to compare it with sildenafil as a positive control.

MATERIALS AND METHODS

Test materials: Mirodenafil (SK3530) was synthesized by SK Chemicals (Suwon, South Korea) with >99.8% purity as determined by both HPLC and potentiometric titrations in glacial acetic acid. The mirodenafil was stable for at least 12 weeks at both ambient temperature and under accelerated conditions (20\(\degree\)C, 75% RH). Sodium nitroprusside (SNP) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). Sildenafil citrate (Viagra\textsuperscript{\textregistered}) was purchased from Pfizer Inc. (Pfizer, New York, NY, U.S.A.). Mirodenafil and sildenafil were dissolved in saline for administration.

Animals: The studies were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” from the National Institute of Health. New Zealand white rabbits (Yonam Laboratory Animals, Cheonan, South Korea) weighing an average of 2.5–3.0 kg were used in this study. The animals were acclimatized for a week and housed individually. During all experiments, the animals were kept in a standard laboratory conditioned room (temperature 23 ± 2\(\degree\)C, humidity range 40–70%, 12 hr light/dark cycle) and were allowed free access to food and UV-sterilized tap water ad libitum.

Surgical procedure for the transection SCI model: Five rabbits per group underwent the same anesthetic management, and an ear vein catheter (22 gauge) was placed to administer additional medications and intravenous fluids. Briefly, the lumbarodorsal fascia region was exposed and cut at the midline of the spinous processes after a dorsal skin incision of the L2-L4 region. The paraspinal muscle was then separated from the spinous process and lamina. Bone extending out laterally from the spinal process was removed with a rongeur (laminctomy). Once the spinal cord was exposed, spinal cord transection was performed using fine cut scissors or a blade between the L2-L3 and L3-L4 region. A single dose of 30 mg/kg of ceftazimine was then injected intramuscularly.

Surgical procedure for the ischemic-reperfusion SCI model: Acute spinal cord injury was induced by the aortic cross-clamping method as described previously [27, 30]. Five rabbits were used in each dosing group. Each animal was anesthetized with an intramuscular injection of xylazine (10 mg/kg) and ketamine (100 mg/kg). An ear vein catheter (22 gauge) was placed for administration of additional medications and intravenous fluids. Heparin at a dose of 150 IU/kg was given intravenously and allowed to circulate for 5 min followed by infusion of 0.9% NaCl solution at a rate of 25 ml/hr. The abdomen was sterilized and draped. A midline laparotomy was performed and the virecta were reflected to the right. The abdominal aorta was then clamped with vascular clamps. One clamp was positioned...
just below the left renal artery and another was placed at the aortoiliac bifurcation. Each animal underwent 40 min of spinal cord ischemia. Collective clamping of the great vessels produced spinal cord ischemia in the rabbits and after 40 min all control animals were rendered paraplegic [9, 22]. Moreover, the reliability and reproducibility of this procedure were good as previously reported [1].

Neurologic examination: Twenty-four and forty-eight hours after the procedures, neurologic status was evaluated using Tarlov’s scoring system.

The criteria were as follows:
- T0, spastic paraplegia with no movement of the hind limbs;
- T1, spastic paraplegia with slight movement of the hind limbs;
- T2, good movement of the hind limbs but unable to stand;
- T3, able to stand, but unable to walk normally; and
- T4, complete recovery.

Penile erection test: Penile erection testing was performed in conscious rabbits [9, 27]. The animals showing a Tarlov’s score of T0 were used for the penile erection test. Penile erection was evaluated at 24 hr after the surgical procedures. After oral administration of either mirodenafil or sildenafil citrate at the dose of 0.3 mg/kg, 1 mg/kg or 3 mg/kg (n=5), the length of the uncovered penile mucosa was measured with a sliding digital caliper. SNP was dissolved in saline (0.2 mg/ml) and administered into the ear vein of the animal at 1 mg/kg 60 min after drug administration. Measurements of the uncovered penile mucosa were made every minute for up to 90 min. The mean length of the exposed penile mucosa in each group was calculated.

Histopathological examination of the spinal cord: The animals were sacrificed at 48 hr after completion of penile erection test and their lumbar spinal cords were removed. The tissue samples were fixed in 10% formalin and embedded in paraffin with a routine follow-up procedure. Sections were cut at a thickness of 4 µm and stained with hematoxylin and eosin and methyl green stains for light microscopic examination. Morphologic evaluation of the spinal cords was done blindly. The histopathological findings were graded from 1 to 3 [21]. Grade 1 indicated the appearance of a normal spinal cord. Grades 2 and 3 denoted swollen axons with occasional necrotic neurons and many necrotic neurons, respectively.

Statistical analysis: Statistical significance was assessed using the Student’s t-test. A value of P<0.05 compared with the control was considered statistically significant.

RESULTS

Effect of mirodenafil on penile erection in the transection SCI model: Mirodenafil or sildenafil citrate (0.3, 1 or 3 mg/kg) was given orally to male rabbits with a surgical transection of the spinal cord, and then their erections were evaluated in a time-course manner by measuring the length of the uncovered penile mucosa every 1 min for up to 60 min. Sixty min after drug administration, SNP was administered into the ear vein of the animal at 1 mg/kg, then measurement of the uncovered penile mucosa was performed every 1 min for up to 30 min.

When given orally, doses of 0.3, 1 and 3 mg/kg mirodenafil induced a penile erection for the first 60 min. The minimal effective dose of mirodenafil was 0.3 mg/kg (Fig. 2). Sildenafil showed no erectile response for the first 60 min at 0.3 and 1 mg/kg. Weak response exposing the uncovered penile mucosa was observed at 3 mg/kg. The onset of erection with mirodenafil (0.3 mg/kg, 12 min; 1 mg/kg, 7 min; 3 mg/kg, 17 min) was faster than with sildenafil (3 mg/kg, 26 min).

When SNP was given intravenously after 60 min, administrations of 1 and 3 mg/kg mirodenafil were significantly potentiated (Fig. 2). Immediately after injection of SNP, both 1 and 3 mg/kg mirodenafil produced a penile erection with a peak mean length of 17.3 ± 2.7 mm and 15.7 ± 2.3 mm, respectively. These effects were clearly stronger than the sildenafil-induced penile erection (3 mg/kg, 4.9 ± 1.1 mm).

Effect of mirodenafil on penile erection in the ischemic-reperfusion SCI model: After oral administration of mirodenafil or sildenafil, penile erections were observed at a dose of 3 mg/kg. However, no other groups showed a response for the first 60 min (Fig. 3). The onset of action for mirodenafil and sildenafil occurred at 5 min and 9 min, respectively. The maximum mean lengths of penile erection at 3 mg/kg of mirodenafil and sildenafil were 4.1 ± 0.6 mm and 3.3 ± 0.4 mm, respectively. After administering SNP, the lengths of penile erections were significantly increased at 1 mg/kg mirodenafil, 3 mg/kg mirodenafil and 3 mg/kg sildenafil. Mirodenafil induced penile erections with maximum mean lengths of 7.5 ± 0.8 mm at 1 mg/kg and 7.1 ± 0.5 mm at 3 mg/kg dose. Sildenafil showed its maximum erec-
tile response of 5.2 ± 0.6 mm at 3 mg/kg. At the same dose, the erectogenic potential of mirodenafil was significantly better than that of sildenafil. Statistical analysis was conducted at the time point of maximum erection in the mirodenafil treated groups. All three doses of mirodenafil were significantly different compared with sildenafil at the p<0.05 level, as indicated by asterisks (*).

Quantification of erectogenic potential of mirodenafil using area under the curve (AUC): The time course of penile erections, expressed as the area under the curve, after oral administration of mirodenafil or sildenafil followed by intravenous injection of SNP is shown in Table 1. The table shows that the erectogenic effect of mirodenafil was potentiated in the transection model. In the ischemic-reperfusion model, oral doses of 0.3 mg or 1 mg/kg alone did not induce a penile erection; co-administration of SNP resulted in a stronger penile erectile response than with SNP alone. A higher dose, 3 mg/kg mirodenafil, alone induced erections and was immediately potentiated by SNP. Oral administration of 3 mg/kg sildenafil alone induced erections in the transection and ischemic-reperfusion models, and co-administration of SNP with 1 or 3 mg/kg of sildenafil induced penile erections that were stronger than SNP treatment alone.

Histopathological examination of the spinal cord: For histopathological examination, the lumbar spinal cords applied mirodenafil or sildenafil were obtained from each rabbit. Neuronal degeneration was observed in the lumbar spinal cords, including shrunken neurons, nissl substance disappearance and neuronal necrosis (Fig. 4). The grades of the lesions were 2 or 3 in all rabbits.

DISCUSSION

Spinal cord injury (SCI) is known as one of the main etiological factors relevant to erectile dysfunction. It has been estimated that 10–19% of ED has a neurogenic origin [14]. Spinal cord injury is one of the main causes of neurogenic ED and results in ED in 50% of cases of lumbar injury. In some men with a spinal injury, only a reflexogenic erection remains [2]. SCI prevents effective release of NO from the parasympathetic nonadrenergic, noncholinergic (NANC) nerves, which prevent the initiation of erection [7].

In this study, the rabbit spinal cord injury model was induced by ischemic-reperfusion and transection method. While in vitro techniques using corpus cavernosal tissues permit initial screening of appropriate pharmacological activity, it is always important to verify that the required activities can be documented in vivo [16]. Penile erection receives afferent information conveyed by somatic and visceral fibers originating from the penis and perigenital area [19]. Furthermore, it receives direct descending projections from a collection of neurons present in the brainstem and hypothalamic nuclei, which are considered premotor neurons. The spinal network that controls penile erection and its relations with afferent fibers from the periphery and descending projections from supraspinal nuclei have only begun to be elucidated [23].

Table 1. Time course of erection response after oral administration of mirodenafil or sildenafil in rabbits with SCI

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Transection model (AUC) mm × min</th>
<th>Ischemic-reperfusion model (AUC) mm × min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–SNP</td>
<td>+SNP</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mirodenafil</td>
<td>0.3</td>
<td>166.24</td>
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<tr>
<td></td>
<td>1</td>
<td>163.87</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>148.47</td>
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<tr>
<td>Sildenafil</td>
<td>0.3</td>
<td>0</td>
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<tr>
<td></td>
<td>1</td>
<td>0</td>
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<tr>
<td></td>
<td>3</td>
<td>13.72</td>
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</tbody>
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a) 0 to 60 min; b) 60 to 90 min; c) 0 to 90 min; d) Time (min) × length of uncovered penile mucosa (mm).
Nitric oxide secreted at the endothelium of vascular origin and nonadrenergic noncholinergic nerve endings traverses the cell membranes of smooth muscle cells within the penile vasculature and trabecular erectile tissues and binds with soluble guanylate cyclase, which converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP). The cGMP in turn facilitates changes in ion channel permeability as well as energy-dependent sequestration of calcium by cellular organelles. Reduced intracellular calcium levels and actin-myosin complex formation are associated with diminished smooth muscle tone. Smooth muscle relaxation within penile arteries and erectile tissues, which elevates regional blood flow and tissue expansion within the corpus cavernosum (CC), is central to development of a penile erection [10, 17]. Cyclic nucleotides, such as cGMP, are inactivated by phosphodiesterases, of which PDE5 is thought to be the predominant form within vascular and erectile smooth muscle tissues [15, 28]. Inhibitors of PDE5, thus, function by potentiating the physiologic erectile response to NO after sexual arousal rather than triggering erection by targeting the PDE5 isoenzyme [29]. These agents amplify the NO-sGC-cGMP signaling pathway underlying ED largely irrespective of etiology.

The NO released from the nonadrenergic noncholinergic neurons is not the only mechanism involved in erection [5]. The fact that endothelial cells constitutively express endothelial nitric oxide synthase (eNOS) and can release NO, which diffuses to adjacent smooth muscle cells and causes them to relax [8], partly supports the reason why mirodenafil alone could induce a penile erection in the SCI model without sexual stimulation or extrinsic NO administration in our study. This suggests that the relaxing action of mirodenafil on the corpus cavernosum prior to administration of SNP may be mediated by the NO released from the endothelium. At the same dose, these effects of mirodenafil alone were clearly stronger than sildenafil alone in inducing penile erection in the absence of an NO-drive.

In this study, the efficacy of mirodenafil was potentiated by intravenous injection of SNP, a nitric oxide donor. Potentiation of the effect by a nitric oxide donor implies that mirodenafil can enhance erectile activity during sexual arousal. Under the experimental conditions in this model, a nitric oxide donor, such as SNP, may replace the naturally released NO and enhance the efficacy of mirodenafil. Although the constitutive release of NO by the penile endothelium may have assisted mirodenafil to cause erection without sexual stimulation or extrinsic NO, the fact that the erectogenic effect of mirodenafil was markedly and immediately potentiated by SNP suggests that mirodenafil may enhance sexual stimulation mediated erection.

As a result, the efficacy of mirodenafil was stronger than that of sildenafil in spinal cord injury models in regard to the peak length of the uncovered penile mucosa and onset time of action. These findings suggest that mirodenafil may be useful for the treatment of erectile dysfunction in patients with a spinal cord injury.

REFERENCES


