

·Original Article·

Experimental study of verapamil on the relaxation of isolated human corpus cavernosum tissues

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Abstract

Aim: To evaluate the relaxant effect of verapamil on human corpus cavernosum *in vitro* and to assess the drug's potential as a treatment for erectile dysfunction (ED). **Methods:** Preparations of the human corpus cavernosum were obtained from recently deceased young men who had had normal erectile function. The isometric tension and detailed curves were recorded when contractions induced by 10 mmol/L phenylephrine were reduced by different doses of verapamil or the vehicle control (sterile water). The tension of human corpus cavernosum preparations are described as a percentage of their top tension before adding verapamil or the vehicle. ANOVA and least significant difference tests were used for statistical analysis. **Results:** Doses of 1 $\mu\text{mol/L}$, 10 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$ verapamil resulted in relaxation of $(35.28 \pm 7.96)\%$, $(55.91 \pm 6.41)\%$, $(85.68 \pm 4.16)\%$ after 30 min, respectively. The vehicle control at the same time point produced relaxation of $(-0.06 \pm 10.57)\%$ ($P < 0.05$). **Conclusion:** Verapamil is significantly effective in relaxing normal human corpus cavernous smooth muscle induced by phenylephrine *in vitro* and the relaxant effect depends on the concentration of verapamil. (*Asian J Androl* 2006 Mar; 8: 195–198)

Keywords: verapamil; erectile dysfunction; penis; calcium channel blocker

1 Introduction

There are many methods currently available to treat erectile dysfunction (ED). Although oral drugs, especially selective phosphodiesterase type-5 inhibitors that led the revolution in the clinical management of ED, have been widely accepted by physicians and patients alike as the first-line therapy, patients who do not respond to

oral pharmacotherapy or who cannot use it are good candidates for intracavernosal and intraurethral treatment as second-line therapy [1]. Several other vasoactive drugs, including calcium channel blockers, were investigated *in vitro* and *in vivo* in animal and/or human studies, and showed varying degrees of relaxant activity in cavernous smooth muscle cells [2–4]. Of these, verapamil is a good candidate for the treatment of ED because of its high efficiency in relaxing cavernosal smooth muscle and low risk of causing significant decreases in blood pressure and increases in heart rate [2, 4].

In our study, considering the quality of tissues, the relaxant effect of verapamil was measured on corpus cavernosum preparations from healthy and relatively young men *in vitro*. The results might help to clarify

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what influence verapamil exerts on normal human corpus cavernosum tissues *in vitro*.

2 Material and methods

2.1 Human corpus cavernosum tissues

Penile tissues for this *in vitro* study were obtained from 12 men aged 23–30 years (mean 26.4 years), who had died accidentally. Before death, each had given permission for any of their tissues to be used for scientific research. It must be emphasized that all human experiments in this study were performed in accordance with the Helsinki Declaration.

All subjects had a history of normal penile erectile function, according to their partners or case histories. Exclusion criteria included those with one of the following disorders: hypertension, diabetes mellitus, atherosclerosis, hyperlipidemia, hypercholesterolemia, renal function insufficiency, gonadal abnormality, endocrine disorders, nervous or mental diseases. Furthermore, the subjects had no history of pelvic operations or irradiation, and no severe injury in the pelvis or external genitalia. Penile amputations were performed within 30 min of the subjects being announced clinically dead.

The excised tissues were immediately placed in chilled modified Krebs' solution and transported to the laboratory where individual corpus cavernosum tissue was dissected into four strip preparations, each measuring approximately $3 \times 3 \times 10$ mm. The duration of dissection was not more than 30 min. All experiments were performed within 12 h of tissue excision.

2.2 Organ chamber studies

The strip preparations were transferred to a 10 mL organ bath chamber containing modified Krebs' solution, which was prepared daily with the following composition: 118 mmol/L NaCl, 25 mmol/L NaHCO₃, 4.7 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 11 mmol/L glucose, 1.5 mmol/L CaCl₂. The bath was maintained at 37°C by a thermoregulated water circuit and continuously bubbled with a mixture of 5% CO₂ and 95% O₂, pH 7.4. The preparations were suspended between two L-shaped metal prongs by means of silk ligatures. One of the prongs was connected to a force transducer (PowerLab/MLT050; AD Instruments, Sydney, Australia) for registration of isometric tension. The other was attached to a movable unit permitting precise adjustment of preload tension. Isometric tension was recorded us-

ing a data acquisition system (ML750 PowerLab/4sp; AD Instruments, Sydney, Australia).

The preparations were given a 90-min period of equilibration. During this time, the tension was regularly adjusted, and a final tension of 5.0 ± 0.1 mN was achieved.

In all experiments, the strip preparations were incubated with 10 µmol/L atropine for 15 min. Each tissue strip was then incrementally stretched for 15 min to the optimal isometric tension, as determined by the maximal contractile response to 10 µmol phenylephrine. Then 10 µL vehicle (deionized sterile water) and three different doses of 10 µL verapamil (0.01 µmol, 0.10 µmol and 1.00 µmol) were added to the four preparations from each individual. The relaxation responses were expressed as the percentage of total relaxation (loss in tone) induced by the addition of 0.1 mmol papaverine HCl to the chambers at the end of the experiment.

2.3 Drugs

The following drugs were tested: verapamil hydrochloride, phenylephrine hydrochloride, atropine, papaverine hydrochloride and components of modified Krebs' solution (all from Sigma-Aldrich, St. Louis, MO, USA). Verapamil was solubilized in deionized sterile water for injection (Solco Basle, Birsfelden, Switzerland).

2.4 Statistical analysis

At 10, 20 and 30 min after addition of verapamil or the vehicle, SPSS 12.0 software for Windows (SPSS, Chicago, IL, USA), one-way ANOVA and least significant difference tests were used to assess statistical significance between relaxant percentages of the vehicle and different doses of verapamil ($P < 0.05$).

3 Results

In the organ bath, the preparations of corpus cavernosum successfully developed contractions when phenylephrine was present. Verapamil did not induce any tone but caused significant relaxation of preparations (Table 1). At each time point, the relaxant effect was significantly dependent on the concentration of verapamil (approximately 1 µmol/L, 10 µmol/L and 100 µmol/L). On the other hand, in the absence of verapamil, the tension of the human corpus cavernosum strips did not show any significant loss until papaverine HCl was added at the end of the experiment.

Table 1. Human corpus cavernosal tension at different time points, described as a percentage of loss in tone (mean \pm SD), after treatment with either increasing doses of verapamil or vehicle, $P < 0.05$, $n = 12$.

Verapamil ($\mu\text{mol/L}$)	Time after treatment (min)		
	10	20	30
0 (vehicle)	-3.15 ± 4.99	-2.87 ± 7.00	-0.06 ± 10.57
1	17.72 ± 8.23	30.15 ± 9.36	35.28 ± 7.96
10	31.34 ± 12.05	49.88 ± 9.38	55.91 ± 6.41
100	52.08 ± 15.88	77.76 ± 9.19	85.68 ± 4.16

4 Discussion

Calcium channels exist widely in the membrane of cardiac, skeletal and smooth muscle cells and neurons. It has been reported that there are *L*-type voltage-gated calcium channels in human smooth muscle of the corpora cavernosum, and contraction of this muscle and detumescence of the penis are highly dependent on an increase in cytosolic concentration of Ca^{2+} [5, 6]. The importance of extracellular and intracellular sarcoplasmic Ca^{2+} stores for penile smooth muscle contraction has been identified [7, 8]. Calcium channel blockers might, therefore, have potential value to deal with elevated smooth muscle tonus in human corpus cavernosum tissue, which is considered a possible cause for chronic erectile failure [9].

As early as 1986, Brindley [10] reported that some drugs known to relax smooth muscle, including verapamil, caused partial erection when injected intracavernosally. In the late 1980s, some *in vitro* animal or human studies tried to clarify the relaxant effect of calcium channel blockers [11,12]. In the early 1990s, especially after the discovery of the important role of the cyclic guanosine monophosphate pathway in the normal mechanism of erectile function, there were fewer studies on calcium channel blockers for treatment of ED. However, as oral agents were soon found to be unable to satisfy every patient, vasoactive drugs for intracavernosal treatment came to the attention of researchers again [2–5]. The potential of verapamil had been clarified in several *in vitro* animal experiments. However, in healthy human corpus cavernosum tissue *in vitro*, the efficacy remains to be confirmed.

The results of the present study indicated that, in human corpus cavernosum tissue, verapamil can successfully relax contraction induced by phenylephrine at

concentrations ranging from $1\mu\text{mol/L}$ to $100\mu\text{mol/L}$. Of note, 30 min after the addition of $1\mu\text{mol}$ verapamil the tension of three human corpus cavernosum strips fell to the level close to total relaxation, which supported the previous findings that high doses of verapamil ($>10\mu\text{mol/L}$) can moderately decrease adrenergic effects [2]. This concentration is much higher than the level of effective blood concentration under the conventional dose for oral or intravenous administration [13], which may be the reason why few cases with improving erectile function have been reported when verapamil has been used for treatment of cardiovascular disease. Therefore, verapamil appears to be a potential candidate as an intracavernosal pharmacotherapeutic agent for the treatment of ED.

Furthermore, compared with intraurethral prostaglandin E1 (MUSE), regarded by many patients with ED as an appropriate and safe treatment, verapamil has an advantage. In the long term, the lack of consistency of the response to MUSE may lower the patient's interest in continuing this treatment [14]. On the other hand, according to the results of this study, the relaxant effect of verapamil always lasted at least 30 min. Under high-dose verapamil, the tension of human corpus cavernosum tissue reduced continuously and became close to the point of total relaxation at 30 min after the drug was added. Therefore verapamil could have a positive impact on the patient's interest in long-term treatment for ED.

In addition to its significant relaxant effect, verapamil has been reported to be able to reduce the risk of priapism when intracavernosal self-injection of papaverine and verapamil was introduced to ED patients [3]. In addition, considering the use of intralesional injection of verapamil to dissolve noncalcified plaque in Peyronie's disease, its antifibrotic activity might prevent fibrosis often caused by intracavernosal injections [15, 16]. Although combined intracavernosal verapamil injection was not confirmed as sufficient to prevent fibrosis in the short term [17], its beneficial histological effect should be further explored on a high-dose, long-term scale.

On normal human corpus cavernosum *in vitro*, verapamil is significantly effective in relaxing contraction induced by phenylephrine, and the effect depends on the concentration of verapamil. Considering the high dose in local tissue and the potential benefits to reduce the risk of complication, verapamil could be a reasonable approach for the treatment of ED, and intracavernosal injection seems to be the most advisable form of administration.

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