Research Paper

Inhibition by tadalafil of contractility of isolated nonpregnant human myometrium

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ABSTRACT

Objective: To investigate the inhibitory effect of tadalafil on the contractility of isolated nonpregnant human myometrium. **Materials and Methods:** The ability of tadalafil (25, 40, and 63 μM) to inhibit 55 mM KCl-induced contractility of isolated nonpregnant human myometrium was studied. The ability of the ATP-sensitive potassium channel blocker glibenclamide (10 μM) and the calcium-sensitive potassium channel (BKCa) blocker iberiotoxin (100 nM) to reverse the inhibitory effect of 40 μM tadalafil on 55 mM KCl-induced myometrial contractility was also studied. **Results:** Tadalafil produced a concentration-dependent inhibition of myometrial contractility that was statistically significant at 40 and 63 μM concentrations of tadalafil. The inhibition by tadalafil of myometrial contractility was statistically significantly reversed by the concurrent administration of glibenclamide and iberiotoxin. **Conclusions:** These results suggest that tadalafil inhibits human myometrial contractility by opening ATP-sensitive potassium channels and BKCa channels. The opening of these channels could have been due to the action of raised intracellular levels of cGMP due to inhibition of PDE-5 by tadalafil. The results suggest that tadalafil could be investigated for use in clinical conditions requiring relaxation of the myometrium.

Key words: ATP-sensitive potassium channels, calcium-sensitive potassium channel channels, relaxant, uterus

INTRODUCTION

Tadalafil is a relaxant of smooth muscle that has been approved by the United States Food and Drug Administration for the management of erectile dysfunction,[1] and pulmonary arterial hypertension.[2] In the first condition, it acts by relaxing the corpus cavernosum and in the second condition by relaxing the pulmonary artery. It is known that tadalafil inhibits phosphodiesterase-5 (PDE-5), the enzyme involved in the catabolism of the second messenger cyclic guanosine monophosphate (cGMP).[1,3] This effect leads to raised levels of intracellular cGMP. Tadalafil has been shown to relax various isolated smooth muscles such as rat prostate,[4]

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human urethra,[5] rat aorta,[6] rat pulmonary arteries,[7] human detrusor,[8] and human ureter.[9] Recently, we showed that the PDE-5 inhibitor sildenafil inhibits the contractility of KCl-induced contractility of the isolated nonpregnant human myometrium.[10] Since tadalafil is a structural congener of sildenafil and these drugs are pharmacologically similar, the objective of the current study was to investigate the inhibitory effect of tadalafil on the contractility of the isolated nonpregnant human myometrium. To date, the inhibitory effect of tadalafil on isolated myometrium has not been studied. It was felt that if tadalafil is found to inhibit myometrial contractility, it could be investigated for potential use in clinical conditions like preterm labor which benefit from myometrial relaxation.

MATERIALS AND METHODS

The study was done in two phases. During the first phase, the inhibitory effects of the vehicle dimethyl sulfoxide (DMSO) and 3 log concentrations of tadalafil (25, 40, and 63 μM) on KCl-induced contractility of the myometrium were investigated. After this, the ability of the reversal agent glibenclamide, an ATP-sensitive potassium channel blocker, to reverse the inhibitory effect of tadalafil on KCl-induced myometrial contractility was investigated. In the second phase of the study, the effect of the solvent used to dissolve tadalafil, DMSO, on the contractility of the isolated myometrium and the ability of the calcium-sensitive potassium channel (BKCa) blocker iberiotoxin to reverse the inhibitory effect of tadalafil on KCl-induced myometrial contractility were studied.

Selection of patients

Patients were chosen from the Division of Obstetrics and Gynaecology, Christian Medical College, Vellore, India. The study's inclusion criteria were nonpregnant women of 20–50 years old undergoing hysterectomy for benign conditions such as fibroids and endometriosis. The study's exclusion criteria were postmenopausal women and patients with malignant conditions. Written informed consent was obtained from each patient included in the study. To make sure, as much as possible, that all myometrial samples used in the study had similar physiological conditions, all myometrial samples were obtained from nonpregnant, nonlactating, premenopausal patients. The study was approved by the Institutional Review Board (IRB Min. No. 8611, dated January 7, 2014).

Tissue preparation

After removing the uterus from each patient, a 2 cm × 2 cm tissue specimen was removed from the lateral wall of the uterus. The specimen was then carried in ice-cold physiological salt solution (PSS) to the Pharmacology Department within 1 h. The uterine specimens were checked using a magnifying glass for muscle fiber orientation. Pathological features like fibroids were also checked for. The serosa and endometrium were dislodged from the specimens. Myometrial strips measuring 10 mm × 3 mm × 3 mm were later made and mounted in a 20 ml organ bath containing PSS kept at a temperature of 37°C and sufficiently aerated with oxygen. The composition of the PSS was in mM: NaCl: 111.5, KCl: 4.6, $MgSO_4$: 1.16, NaH_2PO_4: 1.16, $CaCl_2$: 2.5, $NaHCO_3$: 21.9, and glucose: 11.1. A resting tension of 25 mN (about 2.5 g) was applied to the mounted strip.

Drugs

Potassium chloride (KCl; Qualigens, Mumbai, India), was dissolved in double-distilled water to obtain a concentration of 168 mg/ml. KCl was prepared fresh every day. Tadalafil (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA) to achieve a concentration of 5 mg/ml. Glibenclamide (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in 90% ethanol to obtain a 2 mM stock solution. Iberiotoxin (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in double-distilled water to obtain a 4 μM stock solution.

Experiments involving effect of tadalafil on KCl-induced myometrial contractility and reversal by glibenclamide of inhibitory effect of tadalafil on myometrial contractility

Following an equilibration period of 45 min, the tension was readjusted to 25 mN. The response of the myometrium to the addition of 55 mM KCl was then investigated. 55 mM KCl is the standard concentration of KCl used in our department for stimulating contractility of the isolated myometrium.[10,11] After washing the bath, 55 mM KCl was added again with the test drug tadalafil and the contractile response to KCl was elicited. This way, three log concentrations of tadalafil (25, 40, and 63 μM) were used to investigate the inhibitory effect of tadalafil on KCl-induced myometrial contractility. Then 55 mM KCl was added again, and the contractile response obtained. After washing the bath, 55 mM KCl was added again with 10 μM glibenclamide and 40 μM tadalafil and the contractile response was elicited. This concentration of glibenclamide was the same as that used previously on isolated human myometrium.[11] During each tracing, after drug administration, a contact time of 90 s was given, after which the tissue was washed till the baseline was attained.

Experiments involving effect of dimethylsulfoxide on KCl-induced myometrial contractility and reversal by iberiotoxin of inhibitory effect of tadalafil on myometrial contractility

During the second phase of the study, the following experimental procedure was performed: KCl was administered alone and after washing the bath, KCl was added with 400 μl of the solvent DMSO. After a wash, the contractile response to 55 mM KCl was obtained followed by that to 55 mM KCl and 40 μM tadalafil. After another wash, the myometrial response to KCl alone was obtained followed by the myometrial

response to 40 μM tadalafil, the specific BKCa channel blocker iberiotoxin (100 nM),[12] and 55 mM KCl. The concentration of iberiotoxin used was the same as that used previously on isolated human myometrium.[10]

Analysis of data

Contractility was measured by the maximum height of contraction and the area under the contractile curve (AUCC), a method which we standardized in our department.[10,11] These parameters were calculated by scanning the tracings after each experiment and analysis using the software Image Tool (University of Texas Health Sciences Center, San Antonio, Texas, USA). This was performed by statistically comparing the values of these parameters of the control data (after the administration of KCl alone) and the values of the test data (after the administration of the test drug(s) with KCl). The nonparametric statistical test, Wilcoxon signed rank test, was employed for all statistical analysis on the data.

RESULTS

Nine patients who fulfilled the selection criteria were included in the first phase of the study. Their ages ranged from 40 to 50 years with a mean age of 45 years. Their clinical diagnoses were: Fibroids, six patients; dysfunctional uterine bleeding, two patients; and adenomyosis, one patient. Eleven patients fulfilling the study's selection criteria were included in the second phase of the study. The ages of the patients spanned 36–48 years with a mean age of 42 years. The clinical diagnoses of the patients comprised: Fibroids: Eight patients; and endometriosis: Three patients.

The results of the effect of three concentrations of tadalafil (25, 40, and 63 μM) on 55 mM KCl-induced contractility of the myometrium are shown in Table 1. Tadalafil produced a concentration-dependent inhibitory effect of KCl-induced myometrial contractility which was statistically significant for both height of contraction and AUCC for the 40 and 63 μM concentrations of tadalafil. Glibenclamide significantly reversed the inhibitory effect of tadalafil on myometrial contractility since in its presence, the percent inhibition markedly decreased and became statistically nonsignificant.

The effect of DMSO on KCl-induced contractility of isolated human myometrium is shown in Table 2. DMSO did not significantly inhibit KCl-induced myometrial contractility. Table 2 also shows the effect of iberiotoxin on the inhibitory effect of tadalafil on KCl-induced contractility of the myometrium. 40 μM tadalafil caused a significant inhibitory effect of KCl-induced myometrial contractility, and this was totally and significantly reversed by iberiotoxin. Representative tracings of the effect of tadalafil on KCl-induced myometrial contractility and the reversal effects of glibenclamide and iberiotoxin are shown in Figure 1.

DISCUSSION

This study has shown that tadalafil inhibits the contractility of the isolated nonpregnant human myometrium. Tadalafil at concentrations of 25, 40, and 63 μM produced a concentration-dependent inhibition of myometrial contractility which was statistically significant at 40 and 63 μM concentrations of tadalafil [Table 1 and Figure 1]. Tadalafil is well established to be a smooth muscle relaxant which

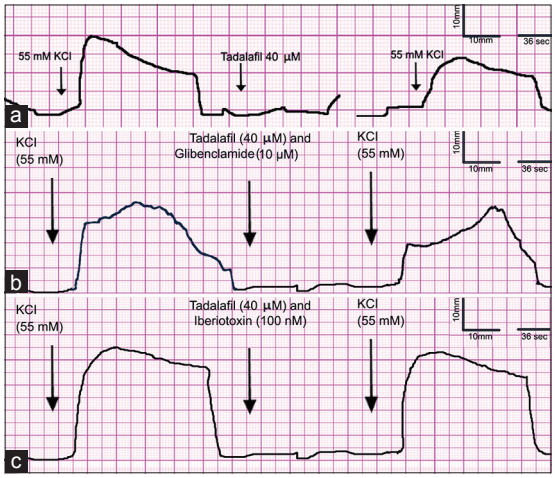

Figure 1: Representative traces from the study: (a) Contractile effect of 55 mM KCl before (left side) and after (right side) addition of 40 μM tadalafil; (b) contractile effect of 55 mM KCl before (left side) and after (right side) addition of 10 μM glibenclamide and 40 μM tadalafil; (c) contractile effect of 55 mM KCl before (left side) and after (right side) addition of 100 nM iberiotoxin and 40 μM tadalafil

Table 1: Dose-dependent inhibitory effect of tadalafil on KCl-induced contractility of isolated human myometrium and reversal of inhibitory effect of tadalafil by glibenclamide (*n*=9 for each drug administered)

Drugs administered	Percentage inhibition of height		Percentage inhibition of AUCC	
	Mean (SEM)	*P*	Mean (SEM)	*P*
55 mM KCl + 25 μM tadalafil	12.94 (3.17)	0.01	15.4 (2.58)	0.06
55 mM KCl + 40 μM tadalafil	26.58 (8.82)	0.01	32.63 (6.33)	0.01
55 mM KCl + 63 μM tadalafil	46.28 (7.17)	0.0001	68.02 (5.55)	<0.001
55 mM KCl + 40 μM tadalafil + 10 μM glibenclamide	19.60 (4.27)	0.0503	22.10 (5.67)	0.14

Values of percent inhibition are compared with those following administration of KCl only. AUCC=Area under the contractile curve, SEM=Standard error of mean

Table 2: Effect of dimethyl sulfoxide on KCl-induced contractility of isolated human myometrium and reversal by iberiotoxin of inhibitory effect of tadalafil on KCl-induced contractility of isolated human myometrium (*n*=11 for each drug administered)

Drugs administered	Percentage inhibition of height		Percentage inhibition of AUCC	
	Mean (SEM)	*P*	Mean (SEM)	*P*
55 mM KCl + DMSO (400 µl)	4.57 (0.197)	0.197	0.83 (6.04)	0.534
55 mM KCl + 40 µM tadalafil	16.57 (5.77)	0.019	26.40 (3.00)	0.001
55 mM KCl + 40 µM tadalafil +100 nM iberiotoxin	−2.38 (8.34)*	0.898	−1.80 (10.17)*	0.700

*Mean values are negative due to the total reversal of the inhibitory effect of tadalafil of KCl-induced myometrial contractility by iberiotoxin. Values of percent inhibition are compared with those following administration of KCl only. AUCC=Area under the contractile curve, DMSO=Dimethyl sulfoxide, SEM=Standard error of mean

is the basis for its clinical use for the treatment of erectile dysfunction and pulmonary arterial hypertension. The finding of the current study indicates that tadalafil has a tocolytic effect, and supports the data of other studies that have shown that tadalafil inhibits the contractility of isolated smooth muscle.[4-9] In our study on the inhibitory effect of sildenafil on the isolated myometrium, it was found that sildenafil significantly inhibited KCl-induced myometrial contractility at concentrations of 3, 10, and 30 µM of sildenafil.[10] In comparison, in the current study tadalafil significantly inhibited KCl-induced myometrial contractility at 40 and 63, but not at 25, µM concentrations of tadalafil [Table 1]. Thus tadalafil appears to be less potent than sildenafil as a myometrial relaxant under *in vitro* conditions. These findings are supported by data on the IC_{50} values of these drugs in previous studies using isolated smooth muscle.[13,14]

In the current study, we found that the ATP-sensitive potassium channel blocker glibenclamide and the BKCa blocker iberiotoxin significantly reversed the inhibitory effect of tadalafil on myometrial contractility [Tables 1, 2 and Figure 1]. Both ATP-sensitive potassium channels and BKCa channels are present in the human myometrium and play a major role in myometrial contractility.[15,16] The mechanism of opening of ATP-sensitive potassium channels and BKCa by tadalafil in our study could have been due to the raised intracellular levels of cGMP due to inhibition by tadalafil of PDE-5. cGMP is known to regulate the gating of ATP-sensitive potassium channels[17,18] and BKCa channels.[19,20]

The current results are supported by the results of previous studies on isolated smooth muscle involving congeners of tadalafil which also act by inhibiting PDE-5, leading to raised intracellular levels of cGMP. In this context, it has been shown that vardenafil-induced relaxation of rat penile arteries was antagonized by the concurrent addition of glibenclamide and iberiotoxin.[21] It has also been shown that the sildenafil-induced relaxation of human urinary bladder dome smooth muscle was antagonized by the concurrent addition of glibenclamide and iberiotoxin.[22] In our earlier study, we found that the inhibitory effect of sildenafil on isolated nonpregnant human myometrium was reversed by iberiotoxin.[10] However, in that study, the reversal effect of glibenclamide on sildenafil's inhibition of myometrial contractility was not investigated.[10]

Preterm labor is an important problem worldwide associated with increased fetal morbidity and mortality, and new and more effective drugs are needed for its management.[23] Tadalafil has a good safety profile in adults receiving it, and to date is not known to cause any major adverse effect to the fetus when administered during pregnancy.[24] Although our findings cannot be directly extrapolated to the pregnant human myometrium, the findings suggest that tadalafil could be useful in the management of clinical conditions that require myometrial relaxation like preterm labor.

The findings of the current study suggest that tadalafil is more potent in relaxing the smooth muscle of the human corpus cavernosum than that of the nonpregnant human myometrium. Thus, in a study on human corpus cavernosum, tadalafil was found to have a relaxant effect even in nanomolar concentrations, with the maximal relaxant effect occurring at a concentration of 10 µM.[25] In comparison, in the current study tadalafil significantly relaxed the nonpregnant human myometrium only at 40 and 63 µM concentrations. A possible reason for this difference is the variation in tissue distribution of PDE-5, with greater concentrations being present in the corpus cavernosum than in the myometrium.[26,27] In the myometrium itself, there are differences in concentrations of PDE-5 between the nonpregnant and pregnant myometrium, with concentrations of PDE-5 rising during pregnancy.[28] Hence, tadalafil could be more potent in relaxing the pregnant myometrium than the nonpregnant myometrium. In this light, our results suggest that investigating the relaxant effect of tadalafil on pregnant human myometrium is warranted. Such an investigation would give a clearer idea of the dose of tadalafil to be used in clinical trials investigating the use of tadalafil for relaxing the myometrium in conditions like preterm labor. Since tadalafil appears to have a more potent relaxant effect on the corpus cavernosum than on the myometrium, the dose of tadalafil for treating preterm labor could be higher than the standard dose used for the treatment of erectile dysfunction (20 mg).[29]

CONCLUSIONS

This study has shown that the smooth muscle relaxant tadalafil inhibits the contractility of the isolated nonpregnant human

myometrium. The results suggest that the inhibitory effect is due to the opening of ATP-sensitive potassium channels and BKCa channels. Tadalafil could be investigated for use in clinical conditions requiring relaxation of the myometrium.

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Conflicts of interest

There are no conflicts of interest.

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