PRODUCTOS NATURALES

Effect of D-004, a lipid extract from royal palm (*Roystonea regia*) fruits, on phenylephrine-induced contractions of isolated rat prostate

Efecto de D-004, un extracto lipídico extraído de las frutas de la palma real (Roystonea regia) sobre las contracciones provocadas por la Fenilefrina sobre la próstata aislada de rata

Lourdes Arruzazabala Valmaña[I]; Rosa Más Ferreiro[II]; Yohani Pérez Guerra[III]; Yazmin Ravelo Calzado[III]; Vivian Molina Cuevas[IV]; Daisy Carbajal Quintana[IV]

[I] Doctora en Ciencias Médicas. Centro de Productos Naturales (CNIC), La Habana, Cuba.
[II] Doctora en Ciencias Biológicas. CNIC, La Habana, Cuba.
[III] Licenciado en Farmacia. CNIC, La Habana, Cuba.
[IV] Doctora en Ciencias Farmacéuticas. CNIC, La Habana, Cuba.

ABSTRACT

D-004, a lipid extract of *Roystonea regia* fruits, inhibits norepinephrine-elicited contraction in rat vas deferens. Nevertheless, the effect of D-004 on α1-adrenergic contractile responses in isolated prostate preparations had not been studied. Therefore, this study investigated the effects of D-004 on phenylephrine-induced contractions in isolated rat prostate strips. D-004 (125-500 mg/mL) was added to prostate preparations in which contractions were induced with phenylephrine (10^{-6}-10^{-4} M) or with ClK (50 mM). D-004 (250-500 μg/mL) significantly ($p < 0,01$).and dose-dependently inhibited the phenylephrine-induced contractions through a non-competitive mechanism, since it reduced (12-60 %) the maximal contraction to phenyle phrine with respect to control preparations. D-004 at 500 μg/mL inhibited similarly both ClK and phenylephrine-contractile responses. Tamsulosin (0.01 μg/mL) abolished (100 % inhibition) the phenylephrine, but unaffected the ClK-induced contractions.

Key words: D-004, isolated prostate, benign prostate hyperplasia, phenylephrine, *Roystonea regia.*

RESUMEN

D-004, un extracto lipídico de las frutas de *Roystonea regia*, inhibe la contracción provocada por la norepinefrina en el vas deferens de las ratas. No obstante, el efecto de D-004 sobre las respuestas contráctiles adrenérgicas α1 en las preparaciones aisladas de la próstata no ha sido estudiado. Por lo tanto, este estudio investigó los efectos de D-004 sobre las contracciones provocadas por la fenilefrina en las extracciones aisladas de la próstata de la rata. D-004 (125-500 μg/mL) fue adicionado a las preparaciones a partir de la próstata en la que las contracciones fueron provocadas por la fenilefrina (10^{-6}-10^{-4} M) o por CIK (50 mM). D-004 (250-500 μg/mL) en forma significativa y la dosis en forma dependiente inhibieron las contracciones provocadas por la fenilefrina mediante un mecanismo no competetitivo, reduciendo así (12-60 %) la contracción máxima por la fenilefrina con respecto a las preparaciones controles. D-004 a 500 µg/mL inhibieron de la misma forma tanto CIK como las respuestas contráctiles-fenilefrina. Tamsulosin (0,01 μg/mL) eliminó la acción de la fenilefrina (100 % de inhibición), pero no afectó las contracciones provocadas por CIK.

Palabras clave: D-004, próstata aislada, hiperplasia benigna de próstata, fenilefrina, *Roystonea regia.*

INTRODUCTION

Urinary outlet obstruction in patients with benign prostatic hyperplasia (BPH) is attributed to the urethral compression produced by the hypertrophied prostatic tissue and to the enhanced stimulation of α_1-adrenoceptors (AR) of both urethral and prostatic smooth muscle, leading to bladder outlet obstruction and lower urinary tract symptoms (LUTS) in patients with BPH.[1] Linked to this fact, a significant increase in the number of α_1-AR in the hypertrophied prostate has been reported.[2] Therefore, α_1-AR-blockers are among the main pharmacological options to treat BPH, mainly effective in relieving LUTS in these patients.[3]

Saw palmetto (*Serenoa repens*) lipid extract (SPLE), which mainly contains fatty acids (oleic, lauric, mirystic, palmitic, among the most abundant) is an herbal drug widely used to treat BPH,[4,5] and although some trials have failed to find differences *versus* placebo,[6] most support its efficacy to treat BPH/LUTS, comparable to that of finasteride and tamsulosin.[7,8] More than a single mechanism contributes to the efficacy of SPLE in BPH, like the inhibition of prostate 5 α-reductase activity,[9] and the antagonism of α_1-AR *in vitro* and *in vivo.*[10,11] Nevertheless, recent studies have shown that SPLE caused indirect α_1-AR-mediated contractions of the rat prostate gland via the release of NE from sympathetic neurons.[12]

D-004, a lipid extract of the royal palm (*Roystonea regia*) fruits that contains a mixture of free fatty acids (oleic, lauric, palmitic and myristic acids as the most abundant), has shown to inhibit competitively rat prostate 5a-reductase activity *in vitro,*[13] and orally given prevented testosterone-induced prostate hyperplasia (PH) in rodents.[14]

As SPLE, D-004 also antagonizes α_1-AR-mediated responses *in vitro* and *in vivo,* since it inhibited markedly and dose-dependently the contractile responses to NE in isolated preparations of rat vas deferens, and orally administered significantly ($p < 0.01$), but modestly, reduced the hypertensive effects induced with NE, and the atypical PH and urodynamic changes induced with phenylephrine (PHE) in the rat.[15-17] Nevertheless, the effect of D-004 on α1-adrenergic contractile responses in isolated prostate preparations had not been studied.

In light of this background, and since prostate is the target organ of BPH and associated LUTS, the aim of this study was to investigate whether D-004 antagonized PHE-induced contractions in isolated rat prostate.

METHODS

Animals

Young adult male SD rats, weighing 250-270 g, were purchased from the National Centre for Laboratory Animals Production (CENPALAB, Havana, Cuba). Animals were adapted to laboratory conditions (temperature 25 ± 3 °C, relative humidity 60 ± 5 %, light/dark cycles of 12 hours) for 7 days. Food (rodent chow obtained from CENPALAB) and water were provided *ad libitum*. Animal handle was conducted in accordance with the Cuban Regulations for the use of laboratory animals and ethical principles for animal management. An independent ethical board approved the use of the animals in the study.

Administration

D-004, obtained from the Chemistry Department of the Centre of Natural Products (Havana City, Cuba), was used after control its composition and purity through validated gas chromatography method. The batch used (021104) had a purity of 91.7 %, as the total of free fatty acids with respect to the raw material (w/w), and the following proportion of each acid: caprilic ($C_{8:0}$) (0.8 %), capric ($C_{10:0}$) (1.0 %), lauric acid (30.2 %), myristic (10.4 %), palmitic ($C_{16:0}$) (7.7 %), palmitoleic ($C_{16:1}$) (0.1 %), stearic ($C_{18:0}$) (2.2 %), oleic ($C_{18:1}$) (29.7 %), linoleic ($C_{18:2}$) (9.5 %), and linolenic ($C_{18:3}$) (0.1 %). D-004 was suspended in a 2 % Tween 65/H_2O vehicle, and suspensions were prepared immediately before use.

In vitro effects on agonist-induced prostate contractions

The animals were anaesthetized with ether. The prostates were then dissected free from extraneous tissue and suspended in an organ bath containing Krebs solution.[12] In this study, the *in vitro* effects were investigated by adding D-004 (125-500 μ/mL) to preparations of rat prostate suspended in organ bath containing Krebs solution assessing 9 sets of experimental conditions.

First, following an equilibration period of 60 min, a control set of experiments in which contractions on rat prostate suspended in organ bath containing Krebs solution with Tween 65/H_2O (negative control) were induced with PHE (10^{-6}-10^{-4} M) added at successive accumulative concentrations or ClK (50 mM). Second, suspensions of D-004 (125, 250 and 500 μg/mL) or tamsulosin (0.001-0.01 μg/mL) (comparison drug) were added to bath solution in independent experiments, and the contractile responses to both PHE and ClK were recorded. Tamsulosin was chosen as comparison drug since this α_{1A}-adrenoreceptor antagonist displays a very effective and potent functional antagonism of NE-induced contractions inhuman prostate *in vitro*.[18]

Contraction of the tissue was recorded isotonically using a lever transducer attached to a Nihon Kohden transducer attached to the Nihon Kohden polygraph.

Statistical analysis

Comparisons between groups were done using the two-sided non-parametric Mann-Whitney U test. The *a priori* level of significance was α= 0.05. All analysis were performed using the software Statistics for Window (Release 4.2; Stat Soft, Inc., USA).

RESULTS

The addition of vehicle, D-004 or tamsulosin did not change the basal tone of the isolated prostate.

Table 1 summarizes the *in vitro* effects on PHE-induced contractile responses in rat prostate strips. Characteristic PHE and CLK-induced contractions were produced in negative control preparations. D-004 (at 250 and 500 μg/mL, not at 125 μg/mL) significantly ($p < 0.01$) and dose-dependently inhibited the contractions induced with PHE in prostate of rat, through a non-competitive mechanism, since D-004 reduced the maximal response to PHE with respect to control preparations. The effect of D-004 was lesser than that of tamsulosin, which at 0.01 μg/mL abolished (100 % inhibition) PHE-induced prostate contractions.

Table 1. Effect of D-004 on contractions induced with phenyleprine (PHE) in isolated rat prostate

Treatment		Magnitude of the contractions (E/Emax) (%)			
Groups	Concentrations µg/mL	1 x 10^{-6} M (PHE)	1 x 10^{-5} M (PHE)	3 x 10^{-5} M (PHE)	1 x 10^{-4} M PHE)
Control N= 8		56.0 ± 4.7	86.1 ± 4.0	100.0 ± 0.0	100.0 ± 0.0
D-004 N= 8	125	46.8 ± 8.3	80.8 ± 9.9	88.2 ± 7.3	88.2 ± 7.3
D-004 N= 8	250	28.1 ± 6.2 +	52.4 ± 12.1+	54.1 ± 11.0 ++	60.0 ± 11.0 ++
D-004 N= 8	500	11.6 ± 1.7 ++	25.4 ± 2.6 ++	40.5 ± 8.2 ++	46.0 ± 10.9 ++
Tamsulosin N= 8	0.001	9.3 ± 6.0 ++	35.6 ± 6.2 ++	72.9 ± 9.9	100.0 ± 0.0
Tamsulosin N= 8	0.01	0	0	0	0

(Mean ± standard deviation). + p <0.01, ++ p < 0.001 Comparison with the control for each agonist concentration (Mann Whitney U test).

The effect of D-004 on rat prostate contractions, however, was not specific, since at 500 µg/mL it inhibited both ClK and PHE-induced contractions in the same extent (table 2). Tamsulosin did not change ClK-induced contractions.

Table 2. Effect of D-004 on contractions induced with KCl (50 mM) in isolated rat prostate

Groups	Concentrations µg/mL	Magnitude of the contractions (E/Emax) (%)
Control n= 8	0	100.0 ± 0.0
D-004 n= 8	250	108.0 ± 15.9
D-004 n= 8	500	43.3 ± 4.09 +
Tamsulosin n= 8	0.01	102.3 ± 3.1

(Mean ± standard deviation). + p < 0,01, Comparison with the control (Mann Whitney U test).

DISCUSSION

The rationale for using α_1-AR blockers to treat BPH/LUTS is based on the physiology and pharmacology of the prostate smooth muscle, since these drugs presumably reduce the resistance along the prostatic urethra by relaxing the smooth muscle component of the prostate. Considering that α_1-AR mediate the contractile response of the prostate and that they are responsible for about 50 % of the prostatic urethral pressure in BPH patients, the use of a treatment able to antagonize α1-AR mediated responses, mainly in the prostate, should be beneficial to manage BPH/LUTS.

In the present study, D-004, a lipid extract from *Roystonea regia* fruits that has demonstrated to inhibit 5α-reductase[13] and to prevent testosterone and PHE-induced PH in rodents,[14] dose dependently and non-competitively inhibited PHE-induced contractions in isolated rat prostate preparations *in vitro.*

Although these results are consistent with previous data supporting the antagonistic effect of D-004 on NE-induced contractions in rat vas deferens preparations,[15] the effect of D-004 on a_1-AR mediated responses in the prostate had not been demonstrated. Considering that recent reports had documented a sympathomimetic effect of SPLE in rat prostate the putative antagonistic effect of D-004 in isolated prostate had to be demonstrated. Thus, despite SPLE had shown to antagonize NE-induced contractions in smooth muscle isolated preparations,[10] a study demonstrated that its addition to isolated rat prostates strips produced an unexpected contractile baseline effect,[12] not reported before. Considering that D-004 shares some similarities in composition and effects with SPLE, the rationale to investigate the effects of D-004 on rat prostate strips was supported. Nevertheless, we found that D-004 unaffected the basal tone of rat prostate, not reproducing the sympathomimetic action described for SPLE. Up to know, we have not explanation for this fact although as free fatty acids are commons for both extracts (D-004 and SPLE) they could not be involved in this sympathomimetic action of SPLE.

The magnitude of the antagonism of D-004 on PHE-induced contractions with D-004, although significant, was moderate, since at 500 mg/mL the response was inhibited by about 60 % compared with the control, being less effective than tamsulosin, which at 0.01 mg/mL abolished the contractile response to PHE, consistent with its potent and effective antagonism of α_1-adrenergic responses in human prostate strips.[18]

In addition, while the antagonistic effect of tamsulosin was specific, since at the dose (0.01 μg/mL) that caused a 100 % inhibition of on PHE-contractions, it unaffected KCl-elicited contractions, the inhibitory effect of D-004 was not specific, since at the highest dose (500 μg/mL) it inhibited both agonist-induced contractions in the same magnitude, suggesting the presence, at this highest concentration of an antispasmodic effects, also referred for SPLE.[19,20]

The lack of specificity of this effect, however, does not necessarily limit its potential relevance on *in vivo* α_1-AR mediated responses, but could reflect the multiple effects derived from the chemical heterogeneity of herbal extracts. Thus, a previous study demonstrated that D-004 (400-800 mg/kg), like tamsulosin (0.05-0.1 mg/kg) significantly and dose-dependently inhibited the volume voided per micturition (VM) reduction in the atypical PH induced with PHE in the rat.[16] This model is useful to assess the effects of α_1-AR antagonists *in vivo*, since the intravenous injection of PHE increases of the prostatic intraurethral pressure and triggers urodynamic parameters, like the reduction of VM, as occurs in patients with BPH. Although not specific, the antagonism of PHE-induced contractions in rat prostate here demonstrated, could explain the *in vivo* antagonistic effect of D-004 on the urodynamics changes induced with PHE.

On the other hand, although high concentrations of D-004 are needed to reach this *in vitro* effect they are in according to D-004 pharmacokinetics studies, where higher concentrations of (3H) oleic acid in rat prostate than in other tissues were founded.[21] In addition, the concentrations of D-004 used in this study were similar than those reported for SPLE.[19,20] The *in vivo* doses of D-004 used in other studies (400-800 mg/kg) are also similar at those reported for SPLE.[22]

CONCLUSIONS

This study demonstrates that D-004 antagonizes PHE-induced contractile responses in rat prostate strips, without affect the basal tone of isolated prostate preparations, but less effectively and specifically than tamsulosin, since at the highest dose D-004 displayed an antispasmodic-like effect, inhibiting also the KCl-induced contractions.

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Dra. C. *Lourdes Arruzazabala Valmañ*a. Centro de Productos Naturales (CNIC). Calle 198 e/ 21 y 19, Atabey, Playa, Apartado Postal 6414, La Habana, Cuba.
Correo electrónico: lourdes.arruzazabala@cnic.edu.cu