Effect of oleic, lauric and myristic acids on phenylephrine-induced contractions of isolated rat vas deferens

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D-004, a lipid extract of *Roystonea regia* fruits that contains oleic, lauric and myristic acids as major components inhibits α1-adrenoreceptors-mediated contractile responses in isolated rat vas deferens and prostate trips; no study has demonstrated a similar effect for oleic, lauric or myristic acids individually. Therefore, the effects of D-004 (250 µg/mL), oleic (100 µg/mL), lauric (50 µg/mL) or myristic (25 µg/mL) acids and their combined effects on phenylephrine (PHE: 10⁻⁷ - 10⁻⁴ mol/L) induced contractions has been studied. No treatment changed the basal tone of the preparations, but all inhibited PHE-induced contractions. D-004 produced the highest inhibition, followed by lauric acid, which was more effective than myristic and oleic acids against PHE-induced contractions of control group. D-004 and the mixture of the three acids produced similar inhibitions.

Keywords: D-004; Oleic, lauric and myristic acids; Phenylephrine induced contractions, Vas deferens

Benign prostatic hyperplasia (BPH), a common disease in the aging men, is a histological condition that consists in an hyperplastic process leading to a measurable growth of glandular-epithelial and stromal/muscle prostatic tissue, which may contribute to the lower urinary tract symptoms (LUTS) common in men with BPH

1, 2. Hormonal factors, like the androgenic hormones testosterone (T) and dihydrotestosterone (DHT), play a crucial role in the etiology of BPH

3, while growth factors and estrogens may contribute to this condition as well. LUTS, however, may or may not be linked with prostate enlargement and bladder outlet obstruction

5. Indeed, urinary outlet obstruction in BPH does not result from the urethral compression produced by the hypertrophied prostate only, but from the enhanced stimulation of α1-adrenoceptors (AR) of both urethral and prostatic smooth muscle

2, 3, 5, being reported an increased number of α1-AR in the prostate of patients with BPH

6. Therefore, α1-AR-blockers are among the first-line pharmacological options to treat BPH, mainly effective in relieving LUTS in these patients

7.

Plant extracts, like saw palmetto lipid extracts (SPLE), are widely used to treat BPH and related LUTS

8-10. Although some trials have failed to find differences versus placebo

11, 12, most studies have supported that saw palmetto is effective to treat BPH/LUTS, with an efficacy comparable to that of finasteride and tamsulosin

13, 14. Multiple, rather than a single mechanism, contribute to the efficacy of SPLE in BPH, like the inhibition of prostate 5 α-reductase activity

15-17 and the antagonism of α1-AR in vitro and in vivo

18, 19.

In addition, major components of SPLE, like lauric, oleic, myristic acids, have been shown to produce similar effects, since lauric acid is a potent inhibitor of 5 α-reductase activity, oleic and myristic acids being also able to inhibit the enzyme activity

15, 20. A recent study also demonstrated that like SPLE, lauric, oleic, myristic and linoleic acids also inhibited the specific binding of radioligands to α1 AR, receptors

21.

D-004 is a lipid extract of the royal palm (*Roystonea regia*) fruits that contains a mixture of free fatty acids wherein oleic, lauric, palmitic and myristic acids are major components. D-004 has been shown to inhibit competitively rat prostate 5α-reductase activity in vitro

22, and orally given prevented T-induced, but not DHT-induced, prostate hyperplasia (PH) in rodents

23-26.

As SPLE, D-004 also antagonizes α1-AR-mediated responses in both in vitro and in vivo conditions. In vitro, D-004 inhibited markedly and dose-dependently
norepinephrine (NE) and phenylephrine (PHE)-induced contractions in isolated preparations of rat vas deferens and prostate strips, respectively\(^ {27,28}\). Also, oral treatment with D-004 to rats significantly reduced the histological features of PH and the urodynamic changes (void volume reduction) induced with PHE\(^ {29,30}\), and modestly, but significantly, attenuated the NE-induced hypertensive effects\(^ {28}\).

To our knowledge, however, no work had demonstrated that weather any of the major components of D-004 can inhibit \(\alpha_1\)-AR-mediated responses. The present study investigated whether the components of D-004 can inhibit PHE-induced contractions in isolated rat vas deferens.

**Animals**—Young adult male SD rats of 9 weeks old, 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°\(\pm\)3°C, 60 ± 5% RH and 12:12 h L:D cycle) for 7 days. Food (rodent chow obtained from CENPALAB) and water were provided ad libitum. Animals handle were maintained 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.

**Chemicals**—D-004 (batch 020907) was obtained from the Chemistry Department of the Centre of Natural Products (Havana City, Cuba). The batch had the following composition (percentages of the relative weight of each acid with respect to that of the raw material): caprilic (C\(_{8}\)O; 0.4%), capric (C\(_{10}\)O; 0.7%), lauric (C\(_{12}\)O; 23.9%), myristic (C\(_{14}\)O; 10.9%), palmitic (C\(_{16}\)O; 10.4%), palmitoleic (C\(_{16}\)O; 0.1%), stearic (C\(_{18}\)O; 2.5%), oleic (C\(_{18}\)O; 32.9%), linoleic (C\(_{18}\)O; 9.1%), and linolenic (C\(_{18}\)O; 0.1%), which was assessed with a validated gas chromatography method. Batch purity (total content of these acids within the raw material) was 91.7%.

D-004 and oleic, lauric and myristic acids (Sigma Chemical CO, St Louis, USA) were suspended in a 2% Tween 65/H\(_2\)O vehicle, and suspensions were prepared immediately before use. Phenylephrine (PHE) was obtained from QUIMEFA, Havana Cuty, Cuba.

**In vitro effects on PHE-induced vas deferens contractions**—The animals were anesthetized with ether. The vas deferens were dissected free from extraneous tissue and mounted in organ baths containing Tyrode solution and bubbled with 5% CO\(_2\) and 95% O\(_2\)\(^ {30}\).

**Experiment 1**

In this experiment, the effects of adding D-004 (250 \(\mu\)g/mL), oleic (100 \(\mu\)g/mL), lauric (50 \(\mu\)g/mL) or myristic acid (25 \(\mu\)g/mL) were observed. The respective concentrations of each acid were equivalent to those present in the added amount (250 \(\mu\)g/mL) of D-004. In brief, following an equilibration period of 30 min, a control set of experiments were conducted. Rat vas deferens preparations were suspended in organ bath containing tyrode solution with Tween 65/H\(_2\)O (control) and contractions were induced with successive accumulative concentrations of PHE (10\(^{-7}\)-10\(^{-4}\) mol/L). Then, suspensions of D-004, oleic or lauric or myristic acid at the concentrations referred above were added to bath solution in independent experiments, and 20 min after the contractile responses to PHE were recorded.

**Experiment 2**

In experiment 2, the effects of D-004 (250 \(\mu\)g/mL) and of combined effect of oleic (100 \(\mu\)g/mL), lauric (50 \(\mu\)g/mL) and myristic acid (25 \(\mu\)g/mL) on PHE-induced contractions in rat vas deferens was studied. Briefly, after a 30 min equilibration period, independent experiments assessed the effects of tyrode solutions containing Tween 65/H\(_2\)O (control), D-004 (250 \(\mu\)g/mL) or oleic (100 \(\mu\)g/mL) + lauric (50 \(\mu\)g/mL) + myristic acid (25 \(\mu\)g/mL) on rat deferens vas contractions induced with successive accumulative concentrations of PHE (10\(^{-7}\)-10\(^{-4}\) mol/L) and of combined effect of oleic, lauric and myristic acids (Sigma Chemical CO, St Louis, USA).

Contractions of the tissues were recorded isotonically using a lever transducer attached to a Nihon Kohden transducer attached to the Nihon Kohden polygraph.

**Statistical analysis**—All values of tissues responses were related to maximal response of control group (E/Emax). Comparison between study groups was performed using the Kruskal-Wallis one-way test. Differences between individual groups and control groups were compared using Dunn’s test. \(P < 0.05\) was considered significant. Statistical analysis were performed using Pathox system software (version 4.2.2; Xybion Corp; Cedar Knolls, NJ, USA).

**Results**

**Experiment 1**—The addition of the vehicle, D-004, lauric, oleic or myristic acids did not change the basal tone of the isolated vas deferens, but D-004 and all the three acids inhibited PHE-induced contractions (Table 1). D-004 (250 \(\mu\)g/mL) and lauric acid (50 \(\mu\)g/mL) significantly inhibited the contractions induced with all the concentrations of PHE (1×10\(^{-7}\)-
Discussion

The results demonstrate that lauric, oleic, and myristic acids, major components of D-004, were able to antagonize PHE-induced contractions on isolated rat vas deferens preparations and that the effect of adding together these three acids at the same relative concentrations present in D-004 were the same as those elicited by D-004 (250 µg/mL).

In addition to inhibit 5α-reductase in vitro and to prevent testosterone-induced prostate hyperplasia in rodents in vivo, D-004 markedly and dose dependently inhibited PHE-induced contractions in isolated vas deferens and rat prostate preparations in vitro.

Although lauric, oleic, and myristic acids, major components of D-004, have been identified as bioactive compounds of SPLE that inhibit prostate 5α-reductase activity, no study had reported if they can inhibit α1-AR-mediated contractile responses. Therefore, in order to know the pharmacologically active constituents of D-004 that contributes to its effects on α1-AR-mediated contractions, the present work was undertaken.

The present study first confirms the inhibitory effect of D-004 on α1-AR-mediated contractions, since the fact that D-004 significantly inhibited PHE-induced contractions of isolated vas deferens is consistent with its effects on nor-epinephrine and PHE-induced contractions in isolated rat vas deferens and prostate preparations, respectively. In addition, it was observed that the addition of lauric (50 µg/mL), oleic (100 µg/mL) and myristic (25 µg/mL) acids also inhibited significantly PHE-induced contractions of rat vas deferens; lauric acid exhibiting the highest inhibition against PHE-induced contractions of control group, showing that the acids, the major components of D-004, are the active compounds of D-004 responsible of its inhibitory effect. Further, the fact that D-004 (250 µg/mL) and the mixture of the three acids at the same concentrations referred above produced a similar inhibition of the PHE-induced contraction confirms this assumption and practically ruled out the possibility of other(s) component of D-004 involved in this effect.

Table 1—Effect of D-004, OA, LA and MA on PHE-induced contractions (Emax, %) of rat vas deferens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PHE concentrations (µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>1 × 10⁻⁷</td>
</tr>
<tr>
<td>Control</td>
<td>1.07 ±0.54</td>
</tr>
<tr>
<td>D004 (250 µg/mL)</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>LA (50 µg/mL)</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>OA (100 µg/mL)</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>MA (25 µg/mL)</td>
<td>0 ± 0.00</td>
</tr>
</tbody>
</table>

PHE= phenylephrine, LA=lauric acid, MA=myristic acid, AO=oleic acid,
P values: ⁰<0.05, ⁰<0.01, ⁰<0.001, ⁰<0.0001; ⁱ<0.05 as compared with control (a-d) and with D-004 (e) (Dunn´ test).

Table 2—Effect of D-004 (250 µg/mL) and the mixture of OA (100 µg/mL), LA (50 µg/mL) plus (MA 25 µg/mL) on PHE-induced contractions (Emax, %) of rat vas deferens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PHE concentrations (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 × 10⁻⁷</td>
</tr>
<tr>
<td>Control</td>
<td>0.8 ± 1.78</td>
</tr>
<tr>
<td>D004</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>OA + LA + MA</td>
<td>0 ± 0.00</td>
</tr>
</tbody>
</table>

PHE= phenylephrine, LA= lauric acid, MA=myristic acid, AO=oleic acid,
*P<0.01 as compared with control (Dunn´ test)
The addition of D-004 or of oleic, lauric and myristic acids did not affect the basal tone of rat vas deferens, which do not reproduce a sympathomimetic effect of SPLE. Thus, SPLE has been shown to antagonize NE-induced contractions in smooth muscle isolated preparations, however, Cao et al. reported that its addition to isolated rat prostates strips produced an unexpected contractile baseline effect, not reported before. Considering that D-004 shares some similarities in composition and effects with SPLE, the effects of D-004 and the three acids on vas deferens basal tone was studied. These results suggest that other component(s) of SPLE different from oleic, lauric or myristic acid is (are) involved in this sympathomimetic effect.

Since α₁-AR mediate the contractile response of the prostate, which is responsible for about 50% of the prostatic urethral pressure in BPH patients, a treatment that antagonizes α₁-AR mediated responses should be useful to manage BPH/LUTS. The present results add to the understanding of the active compounds of D-004 that contributes to the antagonism of α₁-AR blocker-mediated responses, a key mechanism in its potential benefit to manage BPH/LUTS.

Conclusions

The results of the present study demonstrate that the major components of D-004 (oleic, lauric and myristic acids) were active to inhibit the contractile responses induced by PHE in rat vas deferens, and that the effect of D-004 was greater than those of the individual acids, but equivalent to that of the sum of these acids.

References

23. Arruzazabala M L., Carabajal D, Mas R, Molina V, González V & Rodríguez E. Preventive effect of D-004, a lipid extract from Cuban royal palm (Roystonea regia) fruits, on prostate...


