



## Effects of D-004 plus Finasteride on Prostate Hyperplasia Induced with Testosterone in Rats

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**SUMMARY.** Benign prostatic hyperplasia (BPH) is the main cause of lower urinary tract symptoms in older men. Finasteride, a prostate 5 $\alpha$ -reductase inhibitor, is a therapeutic agent widely used for BPH. D-004, a lipid extract from *Roystonea regia* fruits, prevents prostate hyperplasia (PH) induced with testosterone (T), not with dihydrotestosterone (DHT) in rodents. This study was undertaken to evaluate whether the combined therapy with D-004 + finasteride could induce additional benefits on T-induced PH in rats compared with the respective monotherapies. Firstly, we assessed the dose-effect relation of finasteride on this model, for which rats were randomly distributed in six groups (10 rats/group). A negative control group injected with soy oil s.c, and five groups injected with T (3 mg/kg, s.c): a positive control treated only with the vehicle and four groups treated with finasteride (0.5, 1, 3, and 10 mg/kg). Later on, the putative interaction between D-004 and finasteride was investigated, for which rats were randomly allocated in five groups (10 rats/group): a negative control, treated with the vehicle, and four treated with T: a positive control and three groups treated with finasteride (0.5 mg/kg), D-004 (200 mg/kg) and combined therapy D-004 200 mg/kg + finasteride 0.5 mg/kg, respectively. All treatments were administered for 14 days. Bodyweight was controlled weekly. At study completion, prostates were weighed. Finasteride (0.5 - 10 mg/kg) significantly and doses dependently inhibited prostate increase, inhibitions ranging from 45.8% to 100%. Combined therapy inhibited prostate enlargement in 63.7%, the reduction on prostate weight being significant versus the positive control and each monotherapy, which at the doses tested inhibited prostate weight increase by 32.9% (D-004 200 mg/kg) and 30.6% (finasteride 0.5 mg/kg). In conclusion, combined therapy with minimal effective doses of both D-004 and finasteride in this model induced additional benefits in preventing T-induced prostate enlargement compared with each treatment administered alone.

**RESUMEN.** "Efectos de la Terapia Combinada del D-004 y el Finasteride sobre la Hiperplasia de Próstata inducida por Testosterona en Ratas". La hiperplasia prostática benigna (HPB) es la causa principal de los síntomas molestos del tracto bajo urinario en el hombre adulto. El finasteride, un inhibidor de la enzima 5 $\alpha$ -reductasa prostática es un agente terapéutico ampliamente utilizado en la HPB. El D-004, un extracto lipídico obtenido del fruto de *Roystonea regia*, previene la hiperplasia de próstata inducida con testosterona (T) pero no con dihidrotestosterona (DHT) en roedores. Este estudio fue realizado con el objetivo de evaluar si la terapia combinada con D-004 + finasteride puede inducir beneficios adicionales sobre la hiperplasia de próstata inducida por T en ratas comparada con las respectivas monoterapias. Primeramente, se ensayó la relación dosis-efecto del finasteride en este modelo, para lo cual las ratas fueron distribuidas en seis grupos (10 ratas/grupo). Un grupo control negativo inyectado con aceite de soya s.c, y cinco grupos inyectados con T (3 mg/kg, s.c): un grupo control positivo tratado solo con el vehículo y cuatro grupos tratados con finasteride (0.5, 1, 3, and 10 mg/kg). Seguidamente, se investigó la posible interacción entre el D-004 y el finasteride, para lo cual las ratas fueron aleatoriamente distribuidas en cinco grupos (10 ratas/grupo): un grupo control negativo, tratado con el vehículo, y cuatro tratados con T: un control positivo y tres grupos tratados con finasteride (0,5 mg/kg), D-004 (200 mg/kg) y terapia combinada de D-004 200 mg/kg + finasteride 0,5 mg/kg, respectivamente. Todos los tratamientos fueron administrados durante 14 días. Se controló el peso corporal semanalmente. Al finalizar el período de tratamiento, las próstatas fueron pesadas. El tratamiento con finasteride (0,5-10 mg/kg) inhibió de modo significativo y dependiente de la dosis el incremento del tamaño de la próstata, en un rango de 45,8% hasta 100%. La terapia combinada inhibió el crecimiento de la próstata en un 63,7%, siendo significativa con relación al control positivo y a cada monoterapia, las cuales inhibieron el incremento del peso de las próstatas en un 32,9% (D-004 200 mg/kg) y 30,6% (finasteride 0,5 mg/kg). En conclusión, la terapia combinada con dosis efectivas mínimas de D-004 y finasteride en este modelo indujo beneficios adicionales en prevenir el alargamiento de la próstata inducido por T comparado con cada tratamiento administrado de forma separada.

**KEY WORDS:** D-004, Finasteride, Prostatic hyperplasia, Rats.

**PALABRAS CLAVE:** D-004, Finasteride, Hiperplasia prostática, Ratras.

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## INTRODUCTION

Benign prostate hyperplasia (BPH) is the non-malignant and uncontrolled growth of cells and stroma in the prostate gland that lead to difficulty urinating, being the most frequent cause of lower urinary tract symptoms (LUTS) in men over 50 years, its frequency increasing with age<sup>1-3</sup>.

Although the pathogenesis of BPH is not fully understood, a pivotal role is attributed to the hormonal changes occurring in the aging man, like the increased conversion of prostate testosterone (T) in dihydrotestosterone (DHT), a more active metabolite that binds to androgen receptors and increases the release of different growth factors, a reaction catalyzed through the prostate 5 $\alpha$ -reductase enzyme, the excessive accumulation of DHT in the prostate leads to BPH<sup>4,5</sup>. Therefore, the inhibitors of prostate 5 $\alpha$ -reductase are first-choice agents to treat BPH, decreasing prostate enlargement<sup>6,7</sup>.

The prostate contains two 5 $\alpha$ -reductase enzyme isoforms (type 1 and type 2), type 2 being present in high quantities within the prostate<sup>8</sup>. Finasteride, a classical competitive and specific type-2 5 $\alpha$ -reductase inhibitor, is a therapeutic agent widely used to treat BPH that causes atrophy of prostatic glandular epithelial cells and reduces the prostate volume by 20-30%<sup>9</sup>, and can produce a 25% reduction in both obstructive symptom score and peak urinary flow rate, the onset of its effects being slow (3-6 months) but long-lasting<sup>10,11</sup>. The most commonly reported adverse events associated with finasteride are sexual dysfunction, decreased libido, ejaculatory dysfunction, impotence and gynecomastia<sup>12</sup>.

Nevertheless, BPH also involves an increased tone of prostatic smooth muscle under sympathetic innervation. In the human lower urinary tract,  $\alpha$ 1-adrenoceptors mediate smooth-muscle contraction of bladder neck, urethra, and prostate<sup>13</sup>, and  $\alpha$ 1-adrenoreceptors antagonists ameliorate LUTS by inducing smooth muscle relaxation<sup>14-16</sup>.

The combined therapy with prostate 5 $\alpha$ -reductase inhibitor and  $\alpha$ 1-adrenoceptors antagonists reduce the risk of clinical progression of BPH significantly more than did treatment with either drug alone<sup>17,18</sup>.

Phytotherapy has been widely used to treat BPH, mainly the lipid extracts of the fruits of Saw Palmetto, a palm of the Arecaceae family that mainly contain fatty acids, like oleic, lauric, myristic, palmitic, linoleic, capric, stearic and caprylic acids<sup>19-21</sup>.

D-004 is a lipid extract of the fruits of the Cuban palm (*Roystonea regia* - Arecaceae), containing a reproducible mixture of fatty acids, mainly oleic, palmitic, lauric, linoleic, and myristic, followed by palmitoleic, caprylic, capric and stearic acids, which prevents prostate hyperplasia (PH) induced with T, not with DHT, in rodents<sup>22,23</sup>, also showing  $\alpha$ 1-adrenergic blocker effect<sup>24,25</sup>. Up to date, preclinical toxicology has not shown D-004-related toxicity in acute and sub-chronic studies<sup>26</sup>.

This study was undertaken to evaluate whether the combined therapy with D-004 + finasteride could induce additional benefits on T-induced PH in rats compared with the respective monotherapies.

## MATERIALS AND METHODS

### Animals

Male Sprague Dawley rats (250-300 g) were obtained from National Centre for Laboratory Animals (CENPALAB, Havana, Cuba) and adapted to laboratory conditions for 7 days with free access to food and water. All experiences were performed according to the ethical rules for animal management.

### Administration and dosage

D-004 was supplied by the Chemistry Department of the Centre of Natural Products (National Center for Scientific Research, Havana, Cuba), after corroborating its identity and purity by gas chromatography. Finasteride tablets (Merck Sharp & Dohme, Mexico) were used.

D-004 was suspended in Tween 65-water (2%) vehicle, and finasteride tablets were crushed and suspended in acacia gum/H<sub>2</sub>O (10 mg/ml) vehicle. Control animals received equivalent volumes of the Tween 65-water (2%) vehicle. Treatments, included the vehicle, were administered orally by gastric gavage (5 mL/kg).

Testosterone propionate from the Cuban Medical Pharmaceutical Industry (IMEFA, Cuba) was diluted in soy oil and injected subcutaneously (sc) at 3 mg/kg, as described<sup>27</sup>.

### Experimental procedure

Two experiments were performed. Firstly, we assessed the dose-effect relationship of finasteride on this model, for which rats were randomly distributed in six groups (10 rats/group). A negative control group injected with soy oil s.c, and five groups injected with T (3 mg/kg, s.c): a positive control treated only with the vehicle and four groups treated with fi-

nasteride (0.5, 1, 3, and 10 mg/kg), taking into account that doses of finasteride from 1 to 10 mg/kg have been reported as effective in experimental models <sup>28,29</sup>.

The second experiment investigated the putative interaction of D-004 and finasteride on this model, for which rats were distributed in five groups of 10 rats each: a negative control, treated with the vehicle, and four treated with T: a positive control and three groups treated with finasteride (0.5 mg/kg), D-004 (200 mg/kg) and with combined therapy D-004 + finasteride, respectively. The doses selected for the combined therapy scheme were the minimal effective dose of finasteride obtained in the first experiment (0.5 mg/kg) and a submaximal dose of D-004 in this model (200 mg/kg), based in previous data <sup>30</sup>. Treatments were given for 14 days in both experiments. At completion, prostates were weighed.

### Prostate weight

The rats were anesthetized with ether and sacrificed 24 h after the last dosing. The abdomen was opened by incision in middle ventral line, the prostate separated from bladder, removed and weighed.

### Body weight

Body weights were controlled the day before start the treatments and weekly thereafter, the final weight being controlled at the day of the sacrifice.

### Statistical Analysis

Comparisons between treated and control groups were performed using the two-sided nonparametric Mann Whitney U test. The level of statistical significance was set at  $\alpha = 0.05$ . All analyses were performed using Statistics software for Windows (Release 6.0, StatSoft; Inc, USA).

### RESULTS

Table 1 shows the results of dose-effect study of finasteride. Significant increases of prostate weight (PW) and prostate weight/body weight ratio (PW/BW) were observed in the positive control with respect to the negative control group. Finasteride (0.5-10 mg/kg) significantly and dose-dependently inhibited T-induced prostate enlargement (44.4%-100%) and the increase of PW/BW ratio in the rats.

Table 2 lists the results of the experiment assessing the effects of the combined therapy D-004 + finasteride on PH induced with T in the rat. As in the first experiment, PW and PW/BW ratio significantly increased in the positive control with respect to negative control group. Treatment with D-004 (200 mg/kg) and finasteride (0.5 mg/kg) significantly reduced the increases of both PW and PW/BW ratio. Combined therapy inhibited prostate enlargement in 63.7%, the reduction on prostate weight being significant versus the positive control and each monotherapy, which at the doses tested inhibited prostate weight increase by 32.9% (D-004

Treatment	Doses (mg/kg)	PW (mg)	Percent inhibition	PW/BW ( $\times 10^{-3}$ )
Control (-)	0	561.5 $\pm$ 37.5 ***	—	1.38 $\pm$ 0.1 ***
Control (+)	0	883.4 $\pm$ 32.0	—	2.23 $\pm$ 0.07
Finasteride	0.5	735.8 $\pm$ 34.7*	45.8	1.90 $\pm$ 0.11*
Finasteride	1	676.0 $\pm$ 68.2*	64.4	1.74 $\pm$ 0.17*
Finasteride	3	646.8 $\pm$ 61.1*	73.5	1.73 $\pm$ 0.15*
Finasteride	10	550.1 $\pm$ 33.6**	100.0	1.37 $\pm$ 0.12**

**Table 1.** Effects of finasteride on testosterone (T) induced prostate hyperplasia (PH) in rats (X  $\pm$  SE). PW prostate weight, BW bodyweight, SE (standard error). All groups treated with finasteride were injected with T, \* p < 0.05, \*\* p < 0.01 Comparisons with positive controls (Mann Whitney U test).

Treatment	Doses (mg/kg)	PW (mg)	Percent inhibition	PW/BW ( $\times 10^{-3}$ )
Control (-)	0	448.2 $\pm$ 32.4 **	—	1.15 $\pm$ 0.08 **
Control (+)	0	797.4 $\pm$ 26.3	—	2.01 $\pm$ 0.07
D-004	200	682.2 $\pm$ 29.5 * +	32.9	1.77 $\pm$ 0.07 * ++
Finasteride	0.5	690.4 $\pm$ 31.3 * +	30.6	1.78 $\pm$ 0.08 * ++
D004 + Finasteride	200 + 0.5	574.7 $\pm$ 26.8 **	63.7	1.44 $\pm$ 0.05 **

**Table 2.** Effects of D-004 and finasteride on T-induced PH in rats (X  $\pm$  SE) \* p < 0.05; \*\* p < 0.001; Compared *vs.* Control (+), + p < 0.05; ++ p < 0.01 Compared *vs.* combined treatment, (Mann-Whitney U test).

200 mg/kg) and 30.6% (finasteride 0.5 mg/kg). In both experiments, no significant changes of bodyweight were observed in any group compared with the negative controls.

## DISCUSSION

The present study demonstrates that a combined therapy with minimal effective doses of both D-004 and finasteride induced benefits with respect the same doses of both drugs on T-induced PH in rats, since the inhibition induced with such combined therapy (63.7%) was nearly the double than that induced with D-004 (32.9%) or finasteride (30.6%) alone, in an additive, rather than a synergic manner.

Thus, although the etiology of BPH is unknown, it has been associated to hormonal changes occurring in the aging man, mainly related to the unbalance of testosterone and estrogens production, due to the reduction of testosterone production and to the increase of estrogens, all leading to raise the activity of substances that promotes the cellular growth.

Saw palmetto, the phytotherapeutic agent more used to treat BPH <sup>21</sup>, acts through multiple, rather than a single mechanism. Thus, saw palmetto lipid extracts have shown to inhibit the two isoforms (type 1 and 2) of prostate 5 $\alpha$ -reductase <sup>31,32</sup>, but also antagonize  $\alpha$ 1-adrenoreceptors <sup>33</sup>, inhibit cellular proliferation, and display anti-estrogenic and anti-inflammatory effects <sup>20,31,33,34</sup>. As saw palmetto lipid extracts, D-004 has shown more than a single mechanism whereby could be useful for treating BPH, a logical fact considering the similarities in the composition of D-004 and saw palmetto lipid extracts, which justifies to search whether the combination therapy with D-004 + finasteride should produce benefits over the respective monotherapies in the model of PH induced with T in rodents.

The model used in the present study, the T-induced PH in the rat is very dependent of the activity of prostate 5 $\alpha$ -reductase enzyme, being logical that the inhibitors of this enzyme, like finasteride <sup>9-11</sup>, are very effective in this model. Since D-004 prevents T-induced, but not DHT-induced PH in rats <sup>23</sup>, it was supposed that the inhibition of prostate 5 $\alpha$ -reductase was a mechanism involved in the effects of D-004, a fact recently demonstrated <sup>35</sup>. Thus, the fact that combined therapy with minimal effective doses of D-004 and finasteride provokes additive effects in this model is consisting with the presence of

a common mode of action (inhibition of 5 $\alpha$ -reductase) elicited by both treatments.

This result, however, does not discard that the combined therapy with both agents can induce another interactions in men with BPH, where the multiple etiology of the disease and the effects of D-004, including also the antagonism of  $\alpha$ -1 adrenoreceptors <sup>24,25</sup>, support that such interactions can be present, but not in the model used in this study, wherein only responses related with T-dependent prostate enlargement are involved.

Although the effect achieved with the combined therapy could be considered as not relevant, being below the inhibitions induced with finasteride at 3 and 10 mg/kg (73 and 100%, respectively), it should be noted that considering the incidence of adverse effects associated to finasteride administration <sup>11,12</sup>, and the absence of D-004 related toxicity observed up to date <sup>26</sup>, a potential benefit of combined therapy could be based in the use of a lower dose of finasteride for minimising the drug-related adverse events. This subject, however, should be demonstrated in further clinical studies.

Meanwhile, and based in the fact that D-004 shows more than a single effect that could be useful to treat BPH, other experimental studies should explore the putative benefits of administering D-004 with other drugs used to treat BPH, like the  $\alpha$ 1- adrenoreceptors antagonists.

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