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Antagonistic effect of *Lepidium meyenii* (red maca) on prostatic hyperplasia in adult mice

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Summary

The plants from the *Lepidium* gender have demonstrated to have effect on the size of the prostate. *Lepidium meyenii* (Maca) is a Peruvian plant that grows exclusively over 4000 m above sea level. The present study was designed to determine the effect of red maca (RM) in the prostate hyperplasia induced with testosterone enanthate (TE) in adult mice. Prostate hyperplasia was induced by administering TE, and then these animals ($n = 6$, each group) were treated with RM or Finasteride (positive control) for 21 days. There was an additional group without prostate hyperplasia (vehicle). Mice were killed on days 7, 14 and 21 after treatment with RM. Testosterone and oestradiol levels were measured on the last day of treatment. Prostatic stroma, epithelium and acini were measured histologically. RM reduced prostate weight at 21 days of treatment. Weights of seminal vesicles, testis and epididymis were not affected by RM treatment. The reduction in prostate size by RM was 1.59 times. Histological analysis showed that TE increased 2-fold the acinar area, effect prevented in the groups receiving TE + RM for 14 ($P < 0.05$) and 21 ($P < 0.05$) days and the group receiving TE + Finasteride for 21 days ($P < 0.05$). TE increased prostatic stroma area and this effect was prevented by treatment with RM since 7 days of treatment or Finasteride. The reduction in prostatic stroma area by RM was 1.42 times. RM has an anti-hyperplastic effect on the prostate of adult mice when hyperplasia was induced with TE acting first at prostatic stromal level.

Introduction

The prostate is a male reproductive androgen-dependent organ composed of acini, epithelial cells and fibromuscular stroma (Vaughan, 2003). The pathologies of the prostate are very common in adult men, generally caused by an uncontrolled prostatic growth, which could be or could not be malignant. Human prostate hyperplasia has been considered a disease in which stroma is enlarged (Lin *et al.*, 2007), whereas human prostate cancer is associated with the epithelium (Montironi *et al.*, 2007).

The benign prostatic hyperplasia (BPH) is associated with several kinds of symptoms in the lower urinary tract, which are the main cause for patients to start the regular treatment (O'Leary, 2000). In severe cases, bladder outlet obstruction as a consequence of BPH may cause sepsis, bladder damage, renal failure or death (Roehrborn *et al.*, 1999). The incidence of BPH increases with age, affecting almost 50% of men since 50 years old (Roehrborn *et al.*, 1999; Ziada *et al.*, 1999; Marberger *et al.*, 2004). The effect of ageing leads to an increase in the development of symptoms such as bladder obstruction because of the BPH (Vaughan, 2003). Considering that there is a high rate of BPH in humans (Vaughan, 2003), affecting the quality of life in patients when symptomatic, the treatment of this disease is a priority for public health.

There is an association between the use of medicinal plants from the Brassicacea family and the prevention of the different prostate pathologies (Talalay & Fahey, 2001). Plants of the genus *Lepidium* have been demonstrated to

have effects on prostate size. In fact, an integral suspension of *Lepidium latifolium* significantly reduced prostate size and volume in castrated rats where hyperplasia was induced by steroid treatment (Martinez Caballero *et al.*, 2004). More recently, red maca (RM) (*Lepidium meyenii*), a plant that grows over 4000 m in the Peruvian central Andes, was able to reduce the size of the prostate hyperplasia in adult rats (Gonzales *et al.*, 2005, 2007; Gasco *et al.*, 2007). As stroma is involved in the pathogenesis of the human BPH, the present study was designed to determine the effect of RM on prostatic stroma in mice with BPH.

Materials and methods

Animals

Adult mice aged 10 weeks were obtained from the Animal House at the Universidad Peruana Cayetano Heredia. Mice were maintained six per cage at environmental temperature (22 °C). They were fed with Purina laboratory chow and tap water *ad libitum*. For the experiments, they were allocated in six groups, with six mice in each group. Killing of the animals was carried out by the decapitation method. All animal experiments were conducted in compliance with 'Guide for the care and use of laboratory animals' of the National Institutes of Health from the USA (National Research Council, 1996).

Maca extract preparation

Hydroalcoholic extract of RM was prepared with aqueous ethanol (60%, v/v) by percolation at room temperature for 24 h and concentrate at low pressure to constant weight. The extract was prepared by Eng. Alfonso Higa from Agroindustrial Chanchamayo (Lima, Peru). One hundred grams of dried RM hypocotyls produced 13.54 g of hydroalcoholic RM. This extract was further diluted in distilled water to obtain different concentrations in 1 ml. Solutions were placed in vials and kept in a refrigerator at 4 °C until use.

Treatment

Prostate hyperplasia was induced by treatment with testosterone enanthate (TE) given once at day 1 and at day 7 of treatment. The animals were grouped in six different treatment groups, group 1: vehicle group; group 2: TE group; group 3: TE + RM 21 days; group 4: TE + RM 14 days; group 5: TE + RM 7 days; and group 6: TE + Finasteride 21 days.

Group 1 was treated with the vehicle, which only received water (oral route) and an oil solution (i.m. route). Group 2 was treated with TE (0.09 mg g^{-1} body weight) twice (i.m.), once on day 1 and the second one on day 7. Group 3 was treated with TE same as group 2, and simultaneously with RM (140 mg kg^{-1} body weight) daily (oral route) for 21 days (day 1–21). Group 4 was treated with TE same as group 2, and simultaneously with RM daily for 14 days (day 1–14). Group 5 was treated with TE same as group 2, and simultaneously with RM daily for 7 days (day 1–7). Finally, the group 6 was treated with TE same as group 2, and simultaneously with Finasteride (3.6 mg g^{-1} body weight) (positive control) for 21 days (day 1–21).

Organs weight

After animals were killed, several organs [testes, epididymis, ventral prostate (VP) and seminal vesicles] were removed, dissected free of fat and weighted.

Histological studies

Ventral prostate lobes were excised and dissected free of fat. VPs were immersion-fixed in Bouin's solution. After their dehydration, VP was embedded in paraffin. The tissue blocks were sectioned into 5-μm thickness and stained with haematoxylin and eosin (H&E), and then observed under a Leica DM 1000 light microscope coupled to a Leica DFC 290 camera coupled to a personal computer.

In order to analyse the prostatic acini luminar area and stromal area, the images were obtained under a 10× magnification (four fields per prostate). For the epithelial height analysis, the images were obtained under a 40× magnification (four fields per prostate). The epithelial height, acini luminal area and stromal area were assessed using the LEICA APPLICATION SUITE software for Windows.

The prostatic epithelial height (μm) was measured by manually drawing a line through the acinar epithelia (30 measures per field). The acini luminar area (μm^2) was measured by drawing a line around the luminal perimeter and calculating the acini area. The stroma was measured by subtracting the total field area by the total acinar area.

Measurement of serum testosterone and oestradiol

The animals were killed in the morning, between 8 and 10 AM, by decapitation; blood was collected (1 ml) from the cervical trunk. Serum oestradiol and testosterone concentrations were measured by radioimmunoassay using commercial kits (Diagnostic Products Co., Los Angeles, CA, USA); the measurements were done in duplicate. The hormone labelled with iodine-125 was used as radioactive marker. Samples were run in the same assay to avoid inter-assay variation. The intra-assay variation was 6.42%

for oestradiol and 5.5% for testosterone. Sensitivity of testosterone assay was 4 ng dl^{-1} and for oestradiol assay it was 8 pg ml^{-1} (Gonzales *et al.*, 2005).

Total polyphenol assay

Sample

Fifty milligrams of dry maca extract was suspended in 30 ml of water and heated on a water bath for 30 min. Then, it was cooled under running water and transferred quantitatively to a 100 ml volumetric flask, then diluted to 50 ml with water. After settlement of the solids, the liquid was filtered through a filter paper. The first 10 ml of the filtrate was discarded. The next 5 ml of filtrate was diluted to 25 ml with water (sample).

Standard

Fifty milligrams of pyrogallol was dissolved in water and diluted to 100 ml with the same solvent. Five millilitres of this solution was diluted to 100 ml with water. Two millilitres of the sample or the standard solution was mixed with 1 ml of phosphomolybdotungstic reagent and 10 ml of water and diluted to 25 ml with a 29% w/v solution of sodium carbonate. After 30 min, the absorbance was read at 760 nm (A_1 or A_3), using water as a compensation liquid. The next equation was used to calculate the percentage content of polyphenols expressed as pyrogallol:

$$\frac{12.5 \times A_1 \times m_2}{A_3 \times m_1}$$

where m_1 is the mass of the sample that was analysed, in grams; m_2 the mass of pyrogallol, in grams; A_1 the total polyphenols absorbance; and A_3 is the standard absorbance.

Results are expressed as grams of total polyphenols per 100 g of the maca extract. For comparison, it was also used pulverised RM (raw material), and aqueous extract obtained after boiling 120 min the spray-dried hydroalcoholic extract of RM. This will allow to obtain a fraction with polar compounds.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Homogeneity of variance was assessed by Bartlett's test. If variances were homogenous, differences between groups were assessed by analysis of variance (ANOVA). If F-value in the ANOVA test was significant, the differences between pair of means were assessed by the Scheffeé test. If variances were nonhomogeneous, nonparametric tests were used. $P < 0.05$ was considered as statistically significant.

Results

Body weight was similar in the vehicle group and after different treatments. There was no significant difference between the testis weight and epididymis weight in mice treated with TE, TE + RM, TE + Finasteride and vehicle (data not shown). There was a significant difference between the vehicle and the TE group ($P < 0.05$). However, the weight of the seminal vesicle was not different in mice treated with TE, TE + RM and TE + Finasteride ($P > 0.05$).

After treatment with TE, the prostate weight was significantly increased compared with vehicle (19.5 ± 1.24 mg versus 13.0 ± 2.29 mg). Treatment with RM resulted in a reduction of the effect of TE on prostate weight, but it was only significant ($P < 0.05$ compared with TE group), when the mice were treated with RM for 21 days (12.23 ± 0.68 mg). Finasteride administered for 21 days was also able to reduce prostate weight to 12.57 ± 1.45 mg ($P < 0.05$ compared with TE group) (Fig. 1).

The administration of TE increased the prostatic acinar area of mice to $312.62 \pm 36.24 \times 10^3$ μm^2 as expected. However, only mice treated with RM and TE for 14 and 21 days showed a significant decrease ($P < 0.05$) in acinar area to $178.23 \times 10^3 \pm 13.81$ μm^2 and $177.96 \pm 14.48 \times 10^3$ μm^2, respectively, when compared with the TE group (Fig. 2). Finasteride was also capable to reduce the acinar prostate area to $163.36 \pm 14.78 \times 10^3$ μm^2 ($P < 0.05$ compared with TE). Group treated with RM for 7 days did not reduce the prostatic acinar area ($268.66 \pm 18.60 \times 10^3$ μm^2). Figure 3 shows the microphotography of the acinar area from the different treatments.

Prostatic epithelial height measurements were not significantly different between groups ($P > 0.05$) (data not shown). TE increased the prostatic stromal area ($322.41 \times 10^3 \pm 12.59$ μm^2) of mice. In comparison with the TE group, when mice were treated with RM for 7, 14 and 21 days or with Finasteride for 21 days, there was a significant ($P < 0.05$) decrease in the prostatic stromal area to $226.75 \pm 9.11 \times 10^3$, $220.42 \pm 7.91 \times 10^3$, $217.98 \pm 7.17 \times 10^3$ and $203.07 \pm 10.87 \times 10^3$ μm^2 respectively. There were no significant differences between the groups treated with RM or Finasteride (Fig. 4). RM administration did not modify the serum testosterone and oestradiol levels in mice treated with TE (Table 1).

Analysis of polyphenols showed that pulverised RM had 0.16 g polyphenols per 100 g of maca, whereas the hydroalcoholic extract contained 1.63 g polyphenols per 100 g of maca. When the hydroalcoholic extract was boiled in water and freeze-dried the aqueous extract, the amount of polyphenols in the aqueous fraction was 1.98 g polyphenols per 100 g of maca.

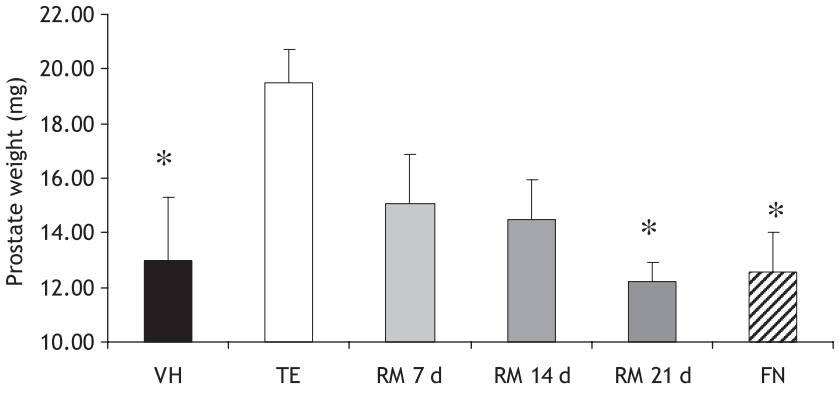

Fig. 1 Prostate weight. Bars indicate mean and standard error of the mean. $*P < 0.05$ compared with TE group. VH: vehicle; TE: treated with testosterone enanthate; RM 7 d: TE + RM for 7 days; RM 14 d: TE + RM for 14 days; RM 21 d: TE + RM for 21 days; Finasteride: TE + Finasteride for 21 days. RM reduced prostate size after 21 days of treatment.

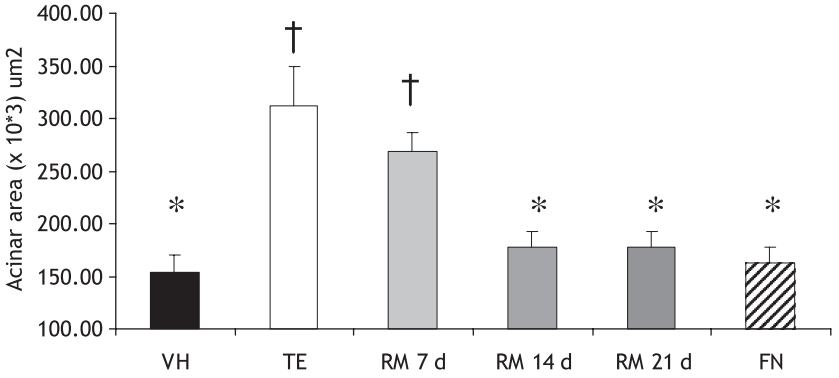

Fig. 2 Prostatic acinar area (μm) in adult mice. $*P < 0.05$ respect to testosterone enanthate group; $^{\dagger}P < 0.05$ compared with the vehicle. VH: vehicle; TE: treated with testoterone enanthate; RM 7d: TE + RM for 7 days; RM 14 d: TE + RM for 14 days; RM 21 d: TE + RM for 21 days; Finasteride: TE + Finasteride for 21 days. RM reduced acinar area since day 14 of treatment.

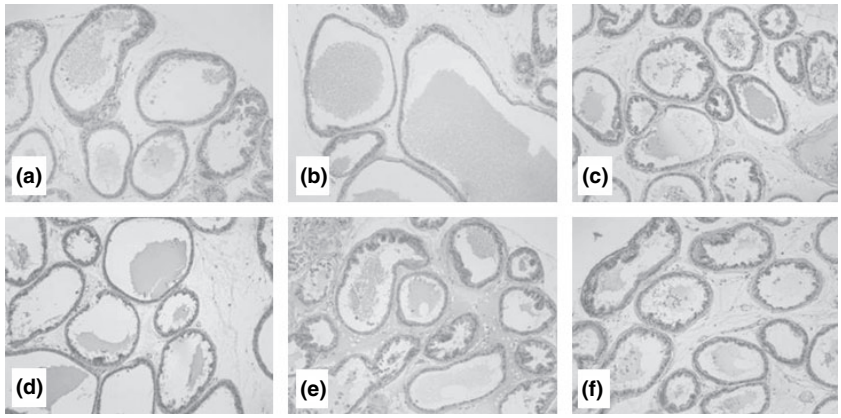

Fig. 3 Microphotography of the prostate acinar area. Treatment with TE (b) increases the prostate acinar area compared with the vehicle (a). Treatment with Finasteride for 21 days (c) significantly reduces the acinar area. Treatment with RM for 7 days (d) has no effect in the acinar area. Treatment with RM for 14 (e) and 21 days (f) significantly reduces the prostate acinar area, being similar as the vehicle group. a: vehicle; b: TE; c: Finasteride; d: RM 7 days; e: RM 14 days; f: RM 21 days. Magnification: 10×.

Discussion

The present study was designed to determine whether the red variety of *L. meyenii* (red maca), a cruciferous plant that grows exclusively over 4000 m in Peruvian central Andes, has effects on epithelial, acini or stroma areas of the adult mice ventral prostate. Previously, we have demonstrated that RM was able to reduce ventral prostate size in rats in which prostatic hyperplasia has been induced with TE. It has been previously demonstrated that the prostate undergoes involution after castration of experimental animals, effect reverted by exogenous administration of androgens (Tsujimura *et al.*, 2002). In this study, we have demonstrated that the model for prostatic hyperplasia using TE might also been reproduced in mice. The dose of TE used and the way of administration (day 1 and day 7 by intramuscular route) was the same as in rats (Gonzales *et al.*, 2005, 2007).

The present study demonstrates that the administration of TE in mice increased the ventral prostate weight at day

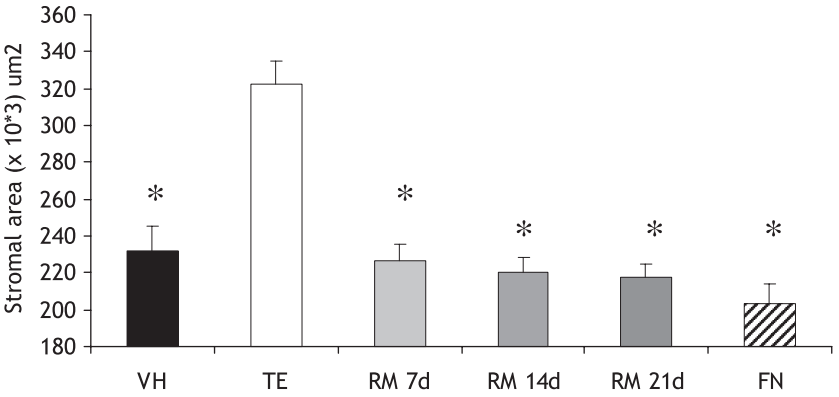

Fig. 4 Prostatic stromal area (μm) in adult mice. *$P < 0.05$ compared with testosterone enanthate group; VH: vehicle; TE: treated with testosterone enanthate; RM 7 d: TE + RM for 7 days; RM 14 d: TE + RM for 14 days; RM 21 d: TE + RM for 21 days; Finasteride: TE + Finasteride for 21 days. Red maca reduced stromal area since day 7 of treatment.

Table 1 Serum testosterone and oestradiol levels in mice treated with vehicle, testosterone enanthate and testosterone enanthate plus red maca by 21 days

Assessment hormone	Vehicle	TE	TE + RM 21 d
Testosterone (ng dl^{-1})	373.6 ± 148.2	1137.9 ± 119.0[a]	1159.1 ± 53.3[a]
Oestradiol (pg ml^{-1})	11.23 ± 7.8	31.5 ± 7.5	25.0 ± 8.7

TE, treated with testosterone enanthate; RM 21 d, red maca for 21 days. Six animals were assessed per group. Data are given as mean ± SEM. [a]$P < 0.05$ with respect to the vehicle group.

21, and increased other androgen-dependent organs like the seminal vesicles. In this model, we can determine that the prostatic hyperplasia was induced by an increase of the prostatic acini and stroma, whereas the prostatic epithelial height was not affected. It is well known from human studies that hyper-proliferative activity of stromal cells is believed to be responsible for the pathogenesis of BPH (Lin *et al.*, 2007).

Epithelial height and luminal areas were proved to be sensitive parameters for the evaluation of androgen effects on the prostates of adult rats (Nishino *et al.*, 2004). The same effect has been observed when castrated rats were treated with testosterone (Nishino *et al.*, 2004) suggesting that the prostatic epithelial is androgen dependent. However, in this study, the prostatic epithelial height was not affected by treatment with TE. It has been previously reported in rats (Gonzales *et al.*, 2005) and mice (present study) that TE produced an increase in prostatic stromal and acini area. In humans, BPH is characterised by an increase in stroma and acini area (Babinski *et al.*, 2003).

Red maca reduced significantly the ventral prostate weight in an effect dependent of time. In fact, prostate size was reduced in a highest level at day 21 of treatment than at day 7; also, its effect was specific for prostate without affecting serum testosterone and oestradiol levels or other androgen-dependent organs like the seminal vesicles, testicles or epidydimis. The specificity of RM on prostate and not seminal vesicles have also been described for adult male rats (Gonzales *et al.*, 2005, 2007).

The luminal areas of prostate acini were significantly reduced, especially in groups treated with RM + TE in days 14 and 21. However, the effect on stromal area was observed as early as 7 days of treatment with RM. This suggests that RM might interfere with the androgen action mainly at the prostatic stromal cells, exerting its effects at a stromal and acini level rather than on epithelial cells. As the increase of stroma and acini area is clearly associated with BPH in men (Babinski *et al.*, 2003), the present findings become of particular importance because RM may be used as an alternative treatment of BPH, particularly in developing countries in which low income resources may difficult the use of conventional medicines.

Maca is characterised by its higher content on aromatic glucosinolates (Li *et al.*, 2001; Dini *et al.*, 2002; Piacente *et al.*, 2002). Recently, it has been described thus a metabolite of the aromatic glucosinolates specifically antagonises androgen receptor (Le *et al.*, 2003). From this, it was suspected that effect of RM on ventral prostate size may be due to glucosinolate metabolites. However, we have recently demonstrated that, after controlling the glucosinolate content, the reduction in prostate weight after treatment with RM was independent of the benzyl glucosinolate content (Gonzales *et al.*, 2007). Moreover, RM obtained as hydroalcoholic extract or aqueous extract has the same effect on reducing prostate weight. This suggests that the action of RM might be located in the most polar fraction of maca. Therefore, it is possible that other compounds may be responsible for the activity of RM.

We have measured the content of total polyphenols in different preparations of RM. Polyphenol content is higher in the hydroalcoholic extract of RM than in the raw material. The amount of polyphenols is further increased when hydroalcoholic extract is boiled with

water, and aqueous extract obtained for this procedure is assessed. Further studies will be required to clarify the mechanism of action of this cruciferous plant. As the effect of hydroalcoholic and aqueous extract of RM are similar on rat prostate size (Gonzales *et al.*, 2007), and that polyphenols are present mainly in the aqueous fraction (most polar fraction), it is suggested that these metabolites could be of interest for further studies looking for the active principle of RM on prostate hyperplasia.

Polyphenols obtained from green tea has been demonstrated to be the inhibitors of 5 alpha-reductase activity (Hiipakka *et al.*, 2002). This property could be of benefit in cases of BPH. Moreover, the presence of polyphenols and flavins in prostatic tissue of humans and mice after green and black tea consumption has been demonstrated (Henning *et al.*, 2006).

From this study, we can conclude that after exogenous treatment with TE on mice, ventral prostate weight is increased, affecting the prostate acini and stroma. This effect can be reverted with RM treatment, which reduces the ventral prostate weight after 21 days of treatment, the prostate acini after 14 days of treatment and the prostate stroma after 7 days of treatment. Therefore, mice may be an ideal animal model for studying BPH, because we can evaluate on them the BPH histological characteristics. Further studies are necessary to clarify the still unknown active principle of RM on ventral prostate and also why its actions are specific as any other organs were significantly affected.

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