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Evaluation of MENT on primary cell cultures from benign prostatic hyperplasia and prostate carcinoma

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Summary

7-alpha-Methyl-19-Nortestosterone (MENT) is a synthetic androgen more potent than testosterone (T) and cannot be reduced at 5-alpha position. No important effects of MENT on prostate growth have been reported. However, little is known about the effect of MENT on benign prostatic hyperplasia (BPH) or prostate carcinoma (CaP). We evaluate the effect of MENT, T and dihydrotestosterone (DHT) on secretion, proliferation and gene expression of primary cell cultures from human BPH and CaP. Moreover, the effect of these androgens was examined in the presence of finasteride to determine the influence of the 5-alpha reductase (5-AR) activity on the androgenic potency. BPH and CaP primary cultures were treated with 0, 1, 10 and 100 nM of T, MENT or DHT during 24 and 48 h. Prostate-specific antigen (PSA) was measured by micro particles immunoassay and proliferation rate by spectrophotometric assay (MTT) and by the immunochemical detection of the proliferation marker Ki-67. Gene expression of FGF8b (androgen sensitive gene) was evaluated by semi-quantitative RT-PCR. Results showed that MENT treatments increased PSA secretion and proliferation rate with a potency ranged between T and DHT. Similar effects of MENT were observed in both BPH and CaP cultures. The studies with finasteride showed that in BPH and CaP cells, the conversion of T into DHT significantly contributes to its effect on the proliferation and PSA secretion, and corroborated the resistance of MENT to the 5-AR. The effect of MENT on the gene expression of FGF8b in CaP cells was similar to T and lower than DHT. It is concluded that MENT increases proliferative and secretory activities and gene expression on pathological prostate cells although in less extent than the active metabolite DHT. Furthermore, the fall of endogenous concentration of T during MENT treatment anticipates that this androgen will be of low impact for the prostate.

Introduction

At present, it is well accepted that the plasmatic level of testosterone (T) declines progressively with age in men, which is associated with a variety of symptoms, affecting life quality. A therapeutic possibility for this condition is the androgen supplementation, which involves high and frequent doses of androgen derivates, usually with secondary effects. For this reason, important research efforts have been focused to obtain an androgen preparation that delivers appropriate and continue doses for long-term therapies.

In animal and human males, the prostate depends on androgens for its growth and function under normal as well as pathological conditions. T and some of its derivates are converted into dihydrotestosterone (DHT) in the prostate by the enzyme 5-alpha reductase (5-AR). DHT is significantly more potent than T in the stimulation of prostate activities. Therefore, the possibility to develop pathological conditions such as benign prostatic

hyperplasia (BPH) or androgen-dependent prostate cancer (CaP) is a risk that must be considered in long-term androgen use, especially in elderly men (Hernandez & Thompson, 2004; Ryan & Small, 2004). There has been considerable interest in developing androgen analogues that stimulate muscle, bone and central nervous system more than prostate. These types of compounds are called selective androgen receptor modulators (SARMs) (Negro-Vilar, 1999). It is believed that SARMs would be suitable for a safer androgen replacement in T-deficient ageing men who are at high risk for developing prostate pathology.

Recently, the 7-alpha-methyl-19-nortestosterone (MENT), a synthetic androgen more potent than T that undergoes no 5-AR has been developed (Kumar *et al.*, 1992). These features make MENT very attractive for replacement therapy and as a male contraceptive agent, because it would not have important effects on the prostate (Noe *et al.*, 1999). However, it is important to determine whether the administration of this androgen has no negative effects on a prostate gland with benign hyperplasia or with an incipient malignancy.

Animal studies indicate that unlike T, the action of MENT is not amplified in the prostate because MENT is not reduced to DHT, probably, because of the presence of a 7-alpha-methyl group interfering with the enzymatic action of 5-AR (Agarwal & Monder, 1988; Kumar *et al.*, 1992). In addition, MENT has been chosen over other androgen analogues for hormone replacement or male contraceptive treatments because of its potency in suppressing gonadotropin secretion (Kumar *et al.*, 1992; Noe *et al.*, 1999) and its ability to be aromatized (LaMorte *et al.*, 1994; de Gooyer *et al.*, 2003). Until now, there is no information about the androgenic potency of MENT in human prostate. The aim of this work was to evaluate comparatively the effects of different doses of T, MENT and DHT at cellular and molecular level, in a primary culture system of BPH and CaP cells. Androgen-sensitive parameters, such as cell proliferation, prostate-specific antigen (PSA) secretion and the gene expression of the fibroblast growth factor 8 and isoform b (FGF8b) (highly androgen-sensitive gene) were evaluated. To our knowledge, this is the first report evaluating MENT effects on human pathological prostate.

Materials and methods

Reagents

All reagents used in this work, unless otherwise indicated, were purchased from Sigma Chemical Co (St Louis, MO, USA). MENT was kindly provided by Population Council (New York, NY, USA).

Prostate tissue

Prostate tissue was obtained from patients undergoing partial (BPH) or radical prostatectomy (CaP) in the Institutional Hospital of the University of Chile under the approval of the Bioethical Committee. Tissue was received in sterile culture medium and processed within 60 min. Some tissue sections were processed by routine histological technique for Gleason classification and control purposes (Gleason & Mellinger, 1974). This procedure allowed us to know the actual classification of the carcinoma samples used for cell isolation. These evaluations were carried out by our institutional pathologists. In this study, five samples of BPH and five samples of CaP with a Gleason score ranged 6–7 were included.

Cell isolation and primary cultures from human prostate samples

Isolation of epithelial cells, and primary cultures were performed according to the method described previously (Castellón *et al.*, 2005). Briefly, small pieces of prostate tissue (1 mm^3) were incubated for 45 min at 37 °C in culture medium to eliminate blood cells. After washing, fragments were digested in collagenase (2.5 mg/mL), hyaluronidase (1 mg/mL) and deoxyribonuclease (0.01 mg/mL), for 2–3 h at 37 °C in a shaking water bath. Every 1 h, dispersed stromal cells were separated from digesting fragments. After this enzymatic digestion, resulting compact and large epithelial cell aggregates were washed with fresh culture medium and further digested with collagenase for another 8–12 h in the same conditions as before. Resulting small aggregates of epithelial cells were mechanically further dispersed, washed and plated in 24-well cell culture plates (Falcon; Becton Dickinson Co, Franklin Lakes, NJ, USA) at a density of 10^6 cells/mL. During the first day of culture, medium (Dulbecco's Modified Eagle's and HAM F-12, 1 : 1) was supplemented with 7% of foetal bovine serum (FBS; Gibco, Invitrogen Co, Carlsbad, CA, USA). After the first change of the medium (2 or 3 days depending on monolayer confluence), cell cultures were washed and fresh medium without FBS but supplemented with human transferrin 5 mg/L, insulin 2 mg/L, epidermal growth factor 10 μg/L, vitamin A and E 200 μg/L, hydrocortisone 10 nM and sodium selenite 2 μg/L, was added.

Morphological characterization of the cultures by immunocytochemistry

Some cells were grown on cover slips, rinsed in PBS and fixed in 3% paraformaldehyde (Merck, Darmstadt, Germany)–2% sucrose in PBS for 30 min at room tem-

perature and blocked with PBS containing 20 mM glycine and 1% bovine serum albumin (BSA). Cell composition of the different cultures was immunocytochemically evaluated using specific rabbit antibodies against CaP tumour antigen PCTA-1 (D-18; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Then, antirabbit-FIT-C (Santa Cruz Biotechnology Inc.) was used to evidence specific antibody binding using a Zeiss Fluorescence Microscope (IM Axioplan Fluorescence Microscope; Carl Zeiss, Göttingen, Germany). PCTA-1 is a specific membrane antigen of prostate epithelial cells, which is significantly overexpressed in malignant tumours (Israeli *et al.*, 1993; Su *et al.*, 1996). Presence of residual stromal cells was evaluated using the same protocol but with specific vimentin antibodies (Santa Cruz Biotechnology Inc.).

Hormonal treatments

To determine the relative effects of hormones on prostate cells, different concentrations (range 1–100 nM) of DHT, T or MENT were added to 70% confluence culture wells. Cultures were exposed to the hormones for 24 or 48 h. Then, media were collected for PSA assay and cells processed for the evaluation of cell growth and RNA extraction.

Determination of proliferative activity

MTT assay

Proliferative activity was assayed using a spectrophotometric assay based on the determination of mitochondrial dehydrogenase activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Carmichael *et al.*, 1987). Briefly, mitochondrial dehydrogenase of viable cells cleaves the tetrazolium ring, yielding purple MTT formazan crystals, which can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. Changes in cell number are directly proportional to absorbance (OD) at 570 nm in a cell-type specific manner. For calibration purpose, separated cultures at different cell densities were assayed by MTT protocol and cell counting. A correlation of cell density vs. absorbance was established.

Immunocytochemistry

Moreover, the expression of the proliferation marker Ki-67 by immunocytochemistry was evaluated. Ki-67 is a nuclear protein, expressed by proliferating cells in all phases of the active cell cycle and it is absent in resting cells. BPH and CaP cells were grown on cover slips and were treated with 100 nM of T, DHT or MENT for 48 h. Then, cells were rinsed in PBS and fixed in 3% paraformaldehyde (Merck)–2% sucrose in PBS for 30 min at room temperature and blocked with PBS containing 20 mM glycine and 1% BSA. The immunocytochemistry was carried out with a specific antigen for Ki-67 (1 : 25) (BD Biosciences, San Jose, CA, USA). For signal amplification and as chromogen, streptavidin-peroxidase and diaminobenzidine were used, respectively.

Determination of PSA

The PSA (seminal glycoprotein from epithelial prostate origin) was used as androgen-sensitive marker of secretory activity of prostate epithelial cells. PSA was determined using a commercial kit based on the biochemical principle of microparticle immunoanalysis (MEIA) [Immunolite/PSA; Diagnostics Products Corporation (DPC), Los Angeles, CA, USA]) (Sanchez de la Muela *et al.*, 1995). Briefly, specific PSA antibodies attached to a glass fibre matrix are bound to sample PSA. Then, a second antibody conjugated with alkaline phosphatase is added. Finally, the chemiluminiscent substrate LSUBX-Immunolite is added and the product is evaluated in automated equipment (Immunolite 1000 systems; DPC). PSA standards of 4.0 and 25 ng/mL were used as controls. Data are expressed as ng of PSA/cells.

Finasteride studies

Finasteride is a synthetic 4-azasteroid compound and is a specific inhibitor of steroid type II 5-AR (Rittmaster, 1997), an intracellular enzyme that converts T into DHT (Moore & Wilson, 1972). BPH and CaP cultures were treated with 0 or 100 nM of T, MENT or DHT in the presence or absence of 200 nM of finasteride (Sigma) during 24 h (in the corresponding cultures, finasteride was added 4 h before androgens). Then, the secretory and proliferative activities were determined as described above.

Determination of FGF8b gene expression

The FGF8b is an androgen-induced growth factor that is overexpressed in prostate cancer (Wu *et al.*, 1997; Tanaka *et al.*, 1998). Moreover, the expression of this growth factor is associated with cancer progression (Dorkin *et al.*, 1999). FGF8b expression was determined by semi-quantitative RT-PCR. CaP primary cell cultures were treated with 100 nM of T, DHT or MENT for 48 h. Total RNA was extracted from the cells by using the Chomczynski solution (Merck). Two microgram of RNA was reverse transcribed by M-MuLV reverse transcriptase (Promega, Madison, WI, USA). Reversed-transcribed products were used for PCR amplification using the following primers (Tanaka *et al.*, 2001); Forward: 5′-CTGCTGTTGCACTTGCTGGTC-3′ and reverse: 5′-GCTCTGCTCCCTCACATGCT-3′. As

internal control, actin was amplified using the following primers (Yamagata *et al.*, 2001); Forward: 5′-TCTACA-ATGAGCTGCGTGTG-3′ and reverse: 5′-TACATGGCT-GGGGTGTTGAA-3′. Amplification was carried out in 25 μL reaction volumes for 30 cycles with denaturation at 94 °C for 1 min, annealing at 65 °C (FGF8b) and 53 °C (actin) for 1 min and extension at 72 °C for 1 min. Amplified products (10 μl) were analysed on a 2% agarose gel and DNA bands were visualized by ethidium bromide staining. The agarose gels were digitally photographed and band intensities were evaluated by SCION IMAGE program (Scion Corporation, Frederick, MD, USA).

Statistical analysis

Non-parametric test of Kruskal–Wallis followed by Dunn's post-test was performed for statistic evaluation. $p < 0.05$ was considered significant. All experiments were repeated at least three times and the results are expressed as mean ± SD.

Results

Cell culture composition

Cultures at the time of treatment showed 85–90% of cells positive for PCTA-1 (specific for epithelial prostate cells and overexpressed in malignant cells). Less than 10% of cells were positive for vimentin corresponding mainly to residual stromal cells.

Effect of MENT, T and DHT on proliferative activity in primary cell cultures from BPH and CaP

Cells were isolated from prostate samples of BPH or CaP tumours that showed similar Gleason score (6–7) in order to obtain equivalent cultures. After the cultures reached 70% of confluence, cells were treated with 0, 1, 10 or 100 nM of T, MENT or DHT during 24 or 48 h. Then, cells were processed for MTT assay. Cell cultures from BPH (Fig. 1A) and CaP (Fig. 1B) increased their proliferation rate under treatments with all three androgen used when compared with untreated cultures. MENT showed a relative potency ranged between T and DHT. Significant differences with T were seen mainly at the highest MENT concentration (100 nM) and after 48 h of treatment. These results were consistent with the qualitative immunocytochemical evaluation of the Ki-67 expression in BPH and CaP cells (Fig. 2). In order to evaluate the influence of the 5-AR of T, 200 nM of finasteride was added to some BPH and CaP cultures during androgen treatments. The results showed that in both BPH (Fig. 3A) and CaP cells (Fig. 3B), the conversion of T

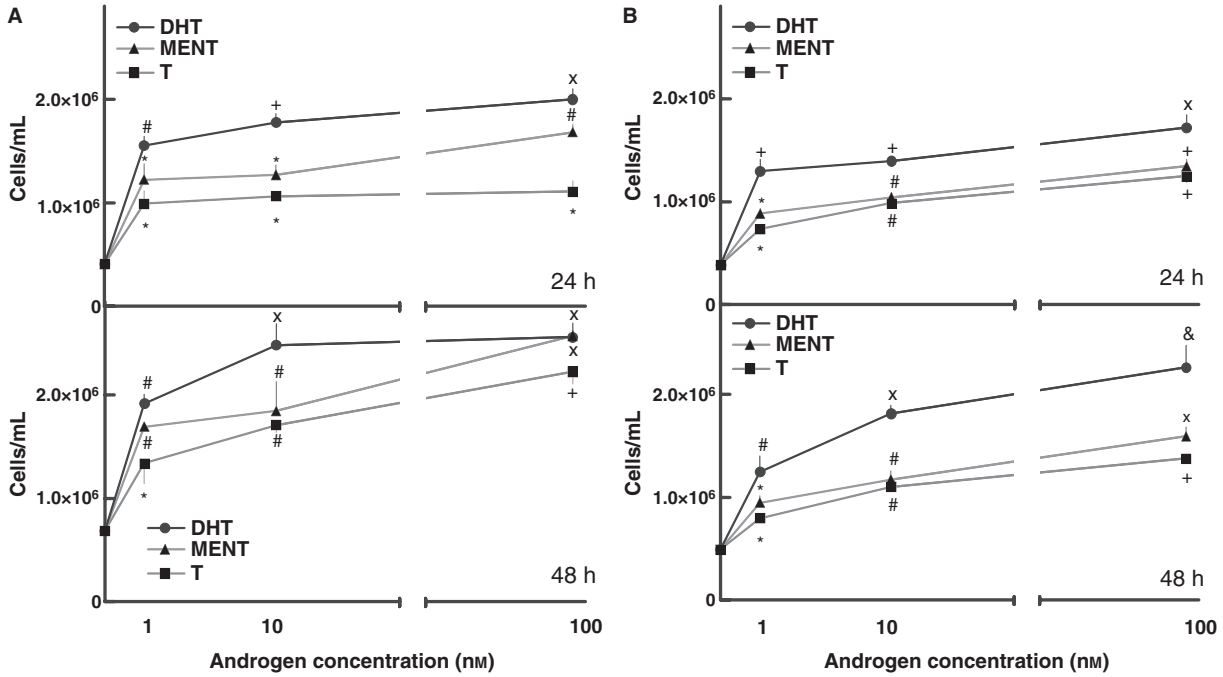

Figure 1 Effect of MENT, T and DHT on proliferative activity in primary cell cultures from BPH and CaP. Cell cultures were treated with 0, 1, 10 and 100 nm of T, MENT or DHT during 24 and 48 h. Then, cells were processed for MTT assay and proliferation rate evaluated. (A) Cultures from BPH. (B) Cultures from CaP. Data are expressed as mean + SD. $N = 5$. All symbols represent statistical significance compared with the control (without androgen). Different symbols indicate statistical significance between groups. $p < 0.05$.

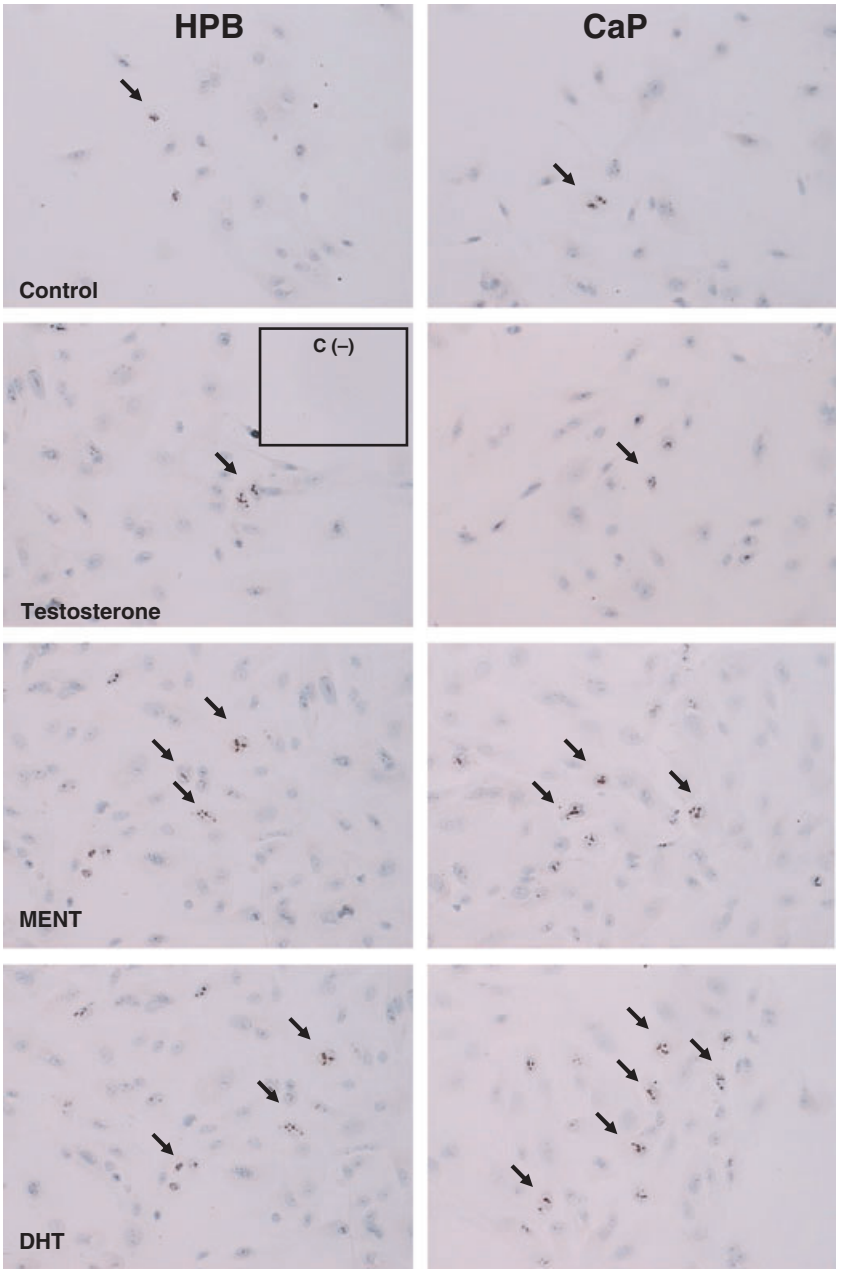

Figure 2 Qualitative immunocytochemistry for the proliferation marker Ki-67. BPH and CaP cells were grown on cover slips and were treated with 0 or 100 nm of T, DHT or MENT for 48 h. The immunocytochemistry was carried out with a specific antibody for Ki-67. BPH cells (left); CaP cells (right). Arrows show positive staining. Negative control without first antibody is shown in the insert.

into DHT contributes to its effect on cell growth (30–35%) and corroborated the resistance of MENT to the 5-AR action.

Effect of MENT, T and DHT on PSA secretion in primary cell cultures from BPH and CaP

To evaluate the effect of T, MENT and DHT on secretory activity of BPH and CaP cells, culture media from the same cultures described before were collected after 24 and 48 h of hormonal treatment for PSA assay. Cultures from BPH (Fig. 4A) and CaP (Fig. 4B) increased their PSA secretion under treatments with all three androgen used compared with untreated cultures. As in proliferative activity, MENT showed a relative potency between T and DHT. Although statistical differences with T were seen at almost all MENT concentrations, the lowest difference was found at 1 nM of MENT. However, the use of finasteride during androgen treatments showed that in BPH (Fig. 5A) and CaP cells (Fig. 5B), the conversion of T into DHT significantly contributes (50–60%) to its effect on the PSA secretion.

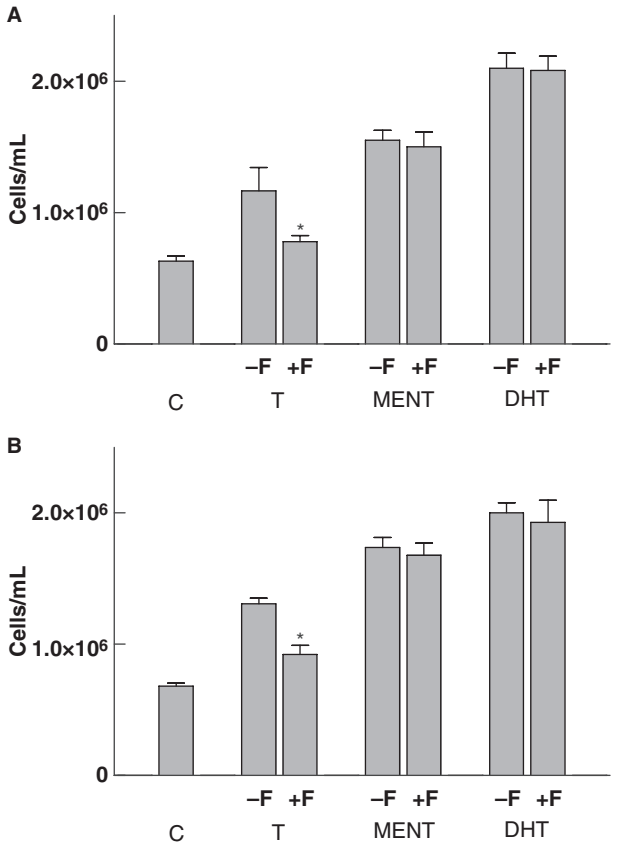

Figure 3 Proliferative activity in the presence or absence of finasteride in primary cell cultures from BPH and CaP. Cell cultures were treated with 0 or 100 nm of T, MENT or DHT during 24 h in the presence or absence of 200 nm of finasteride. Then, cells were processed for MTT assay and proliferation rate evaluated. (A) Cultures from BPH. (B) Cultures from CaP. Data are expressed as mean + SD. $N = 3$. The asterisk indicates statistical significance. $p < 0.05$.

Effect of MENT, T and DHT on FGF8b gene expression in primary cell cultures from CaP

In order to evaluate the direct effect of T, MENT or DHT on gene expression, the level of FGF8b mRNA was evaluated. FGF8b gene was selected because its expression is highly controlled by androgen. In addition, this growth factor is expressed specifically in prostate and it has been described as a marker for malignant progression in CaP. For these experiments, cells were isolated only from CaP tumour samples (Gleason score 6–7). After the cultures reached 70% of confluence, cells were treated with 100 nM of T, MENT or DHT. After 48 h, total RNA was extracted and reverse transcription was carried out. The reversed transcribed products were used for PCR amplification. Cultures from CaP increased their FGF8b mRNA expression under treatments with all three androgen used compared with untreated cultures. However, DHT showed the highest effect while MENT and T exhibited similar potency (Fig. 6). Interestingly, untreated cell cultures showed high based level of FGF8b mRNA.

Discussion

The use of androgen analogues as replacement therapy and in male contraceptive treatment has been widely discussed in the last few years (Sundaram *et al.*, 1993, 1994; von Eckardstein *et al.*, 2003). Potency, half-life, absorption, metabolism and androgen/anabolic ratio have been considered of most importance for the development of new analogues (Walton *et al.*, 2007). However, no studies on the effects of these androgen derivates on human prostate function, at cellular and molecular level, have been carried out. Especially in the older men, this is an extremely important issue that present a high prevalence of usually undiagnosed prostate pathology (Hernandez & Thompson, 2004; Ryan & Small, 2004). DHT is the active metabolite in the prostate; therefore, non-reducible analogues represent important therapeutic options.

7-alpha-Methyl-19-Nortestosterone, a synthetic non-reducible androgen, has been evaluated in a variety of species both *in vitro* and *in vivo*, and also in men, as a male contraceptive (Kumar *et al.*, 1997; Suvisaari *et al.*, 1999; von Eckardstein *et al.*, 2003), and as a replacement therapy in men with androgen deficiency (Sundaram *et al.*, 1993, 1994; Anderson *et al.*, 1999, 2003). In addition, MENT has high affinity for the androgen receptor and low affinity for sex hormone binding globulin, the major carrier serum protein (Kumar *et al.*, 1999). Presently, MENT has been considered over other androgen analogues for androgen replacement therapy or male contraception treatment because of its potency in suppressing gonadotropin secretion (Kumar *et al.*, 1992; Noe *et al.*, 1999), ability to be aromatized (LaMorte *et al.*, 1994; de Gooyer *et al.*, 2003), and inability to be reduced at 5-alpha position (Kumar *et al.*, 1992; Agarwal & Monder, 1988). Although these characteristics may favour actions of MENT on tissues other than prostate, there is no direct evidence, at cellular or molecular level, of possible MENT effects on human hyperplasic or malignant prostate. Recently, using a mice transgenic model of prostate cancer, MENT showed a more potent effect than T on prostate growth (Shao *et al.*, 2006).

In the present work, we have comparatively evaluated the effects of different concentrations of MENT, T and DHT on proliferative activity, PSA secretion and gene expression in primary cell cultures from BPH and CaP. Our results showed that MENT has an effect on prostate proliferative activity similar to T and lower than DHT, when the three androgens are compared in the absence of finasteride. Only high MENT concentration (100 nM)

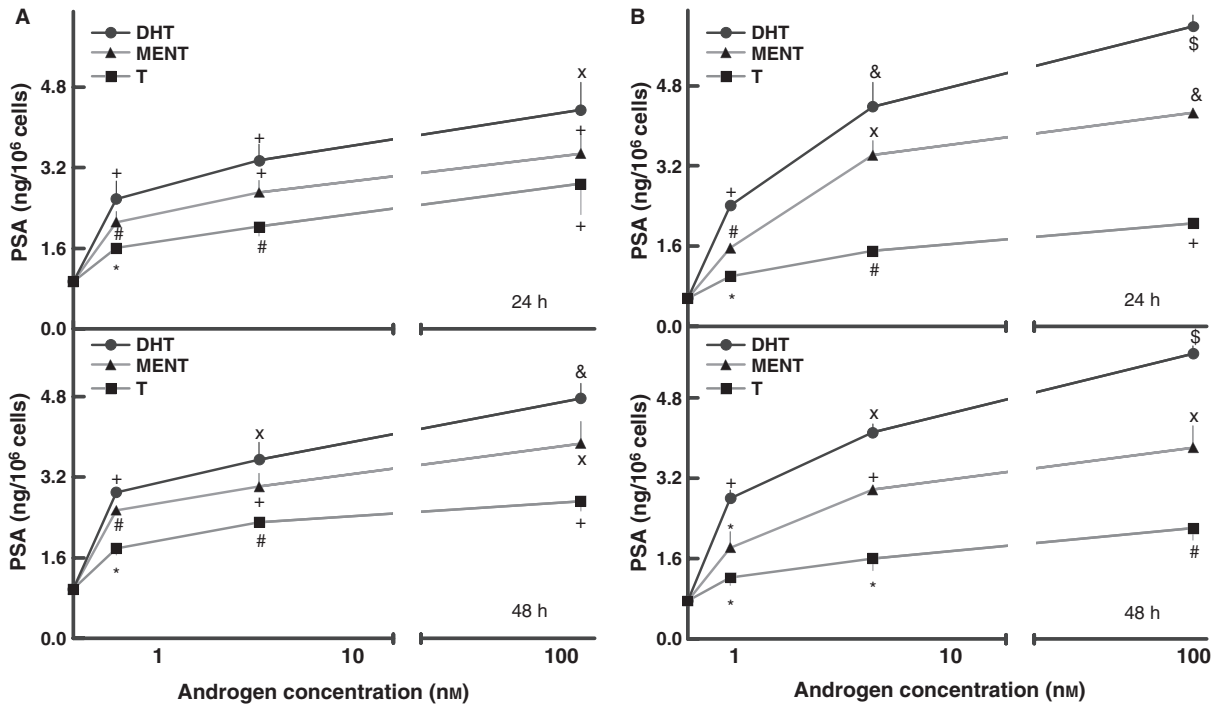

Figure 4 Effect of MENT, T and DHT on PSA secretion in primary cell cultures from BPH and CaP. Cell cultures were treated with 0, 1, 10 or 100 nm of T, MENT or DHT during 24 and 48 h. Then, culture media were collected for PSA assay. (A) Cultures from BPH. (B) Cultures from CaP. Data are expressed as mean + SD. $N = 5$. All symbols present statistical significance compared with the control (without androgen). Different symbols indicate statistical significance between groups. $p < 0.05$.

showed significantly higher stimulatory effect than T. These results correlate with those seen in primates in which MENT was 10 times more potent than T on gonadotropin suppression and stimulation of anabolism, but only two times more potent than T in stimulating the prostate (Kumar *et al.*, 1992, 1997; Sundaram *et al.*, 1993, 1994). A possible explanation for these differences might be accounted by the recruitment of different co-regulator molecules in the different tissues. Considering that effective therapeutic concentration of MENT is approximately 1.4 nM (Anderson *et al.*, 1999, 2003), our results suggest that at this dose, MENT would not have significant effect on the growth rate in pathological prostate.

7-alpha-Methyl-19-Nortestosterone also has an intermediate effect between T and DHT on the PSA secretion in BPH and CaP cell cultures. However, the main differences with T are seen between 10 and 100 nM, especially in CaP cells. Comparing androgen effect on growth and PSA secretion in prostate cells is a very important issue because it has been reported that both activities are differentially regulated by T (Luke & Coffey, 1994; Lee *et al.*, 1995), probably by genomic and non-genomic mechanisms. Moreover, several pharmacological agents, including some that bind to androgen receptor, may have opposite effects on cell proliferation and PSA secretion (Dixon *et al.*, 2001).

In order to evaluate the magnitude of the effect of conversion of T into DHT in the pathological prostate, we used the specific inhibitor of the 5-AR, finasteride. Our results showed that this conversion contributes significantly on the effect of T on the proliferative and secretory activity in BPH and CaP cells. These results indicate that activity of 5-AR is well conserved in pathological prostate. Interestingly, the impact of reduction of T into DHT is significantly higher on PSA secretion than on proliferation rate, both in BPH and CaP cell cultures. These results are consistent with the observation that DHT at high concentration has a more potent stimulatory effect on PSA secretion than on growth rate in prostate-derived cell lines. Increase in growth, but not in PSA secretion, occurs at low doses of DHT (Lee *et al.*, 1995).

Another important issue to be evaluated in androgenic compounds, is their capacity of directly regulating specific genes' transcription. To evaluate this aspect, we chose FGF8b because the expression of this gene is highly androgen sensitive. Also, FGF8b protein expression is correlated with the progression of the prostate cancer malignancy (Tanaka *et al.*, 1998; Dorkin *et al.*,

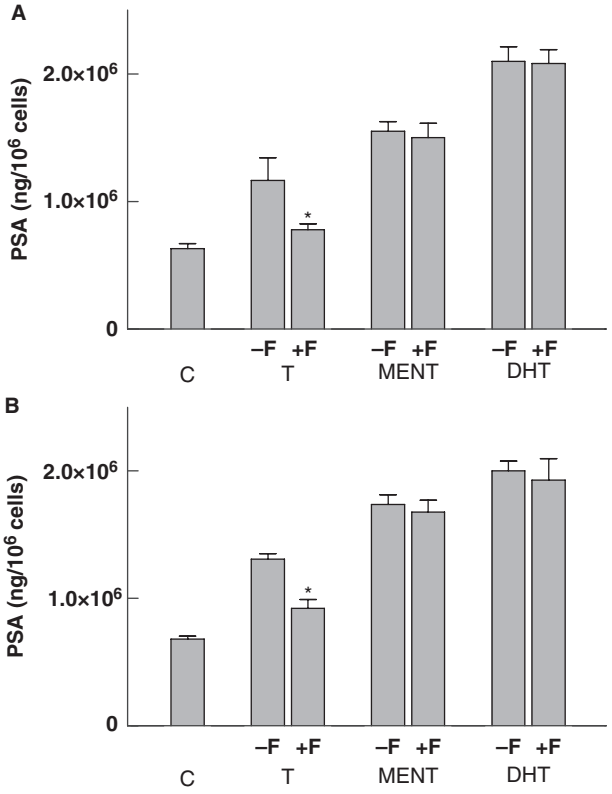

Figure 5 Secretory activity in the presence or absence of finasteride in primary cell cultures from BPH. Cell cultures were treated with 0 or 100 nm of T, MENT or DHT during 24 h in the presence or absence of 200 nm of finasteride. Then, culture media were collected for PSA assay. (A) Cultures from BPH. (B) Cultures from CaP. Data are expressed as mean + SD. $N = 3$. The asterisk indicates statistical significance. $p < 0.05$.

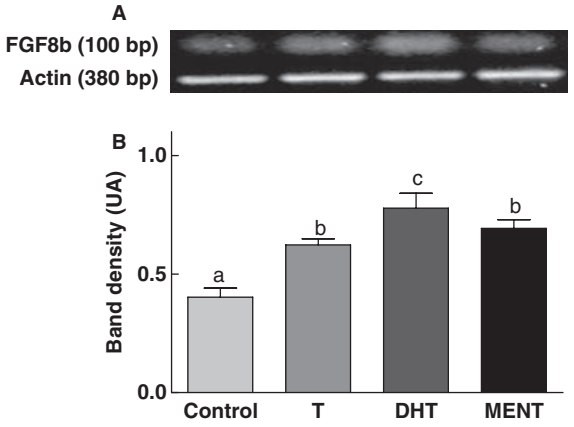

Figure 6 Effect of MENT, T and DHT on gene expression in primary cell cultures from CaP. Cell cultures were treated with 0 or 100 nm of T, MENT or DHT during 48 h. Then, total RNA was extracted and 2 μg of RNA was reverse transcribed. Products were used for PCR amplification. Data correspond to three independent experiments, using cells cultures from prostates with similar histopathological features. (A) FGF8b vs. actin digitalized image corresponding to a representative experiment. (B) Densitometric analysis of the bands. The density is expressed as arbitrary units (AU). Data are expressed as mean + SD. $N = 3$. Different superscripts indicate statistical significance. $p < 0.05$.

1999). Our results showed that untreated CaP cells have a basal expression of FGF8b, suggesting the presence of cells with androgen receptors constitutively activated or mutated in a way that can be activated by different ligands. T and MENT showed similar stimulatory effect on FGF8b gene expression, while DHT exhibited the highest potency. As treatment with androgens was carried out in the absence of 5-AR inhibitor, T induction of gene expression could be partially mediated by its conversion to DHT.

The results obtained in this work suggest that MENT may be considered as an alternative agent for the androgen therapy, because although it can stimulate prostate activities, important effects are seen only at doses over 10 nM, while effective therapeutic concentration are reached at 1.4 nM (Anderson *et al.*, 1999, 2003). However, clinical studies to evaluate the *in vivo* prostate concentrations of MENT and T during MENT administration are needed.

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References

Agarwal, A. K. & Monder, C. (1988) *In vitro* metabolism of 7-alpha-methyl-19-nortestosterone by rat liver, prostate and epididymis. *Endocrinology* 123, 2187–2193.

Anderson, R. A., Martin, C. W., Kung, A. W., Everington, D., Pun, T. C., Tan, K. C., Bancroft, J., Sundaram, K., Moo-Young, A. J. & Baird, D. T. (1999) 7-alpha-methyl-19-nortestosterone maintains sexual behavior and mood in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism* 84, 3556–3562.

Anderson, R. A., Wallace, A. M., Sattar, N., Kumar, N. & Sundaram, K. (2003) Evidence for tissue selectivity of the synthetic androgen 7-alpha-methyl-19-nortestosterone in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism* 88, 2784–2793.

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. & Mitchell, J. B. (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. *Cancer Research* 47, 943–946.

Castellón, E., Venegas, K., Sáenz, L., Contreras, H. & Huidobro, C. (2005) Secretion of prostatic specific antigen, proliferative activity and androgen response in epithelial–

stromal co-cultures from human prostatic carcinoma. *International Journal of Andrology* 28, 39–46.

Dixon, S. C., Knopf, K. B. & Figg, W. D. (2001) The control of prostate-specific antigen expression and gene regulation by pharmacological agents. *Pharmacological Review* 53, 73–91.

Dorkin, T., Robinson, M., Marsh, C., Bjartell, A., Neal, D. & Leung, H. (1999) FGF8 over-expression in prostate cancer is associated with decreased patient survival and persists in androgen independent disease. *Oncogene* 18, 2755–2761.

von Eckardstein, S., Noe, G., Brache, V., Nieschlag, E., Croxatto, H., Alvarez, F. *et al.* (2003) International Committee for Contraception Research, The Population Council, a clinical trial of 7-alpha-methyl-19-nortestosterone implants for possible use as a long-acting contraceptive for men. *Journal of Clinical Endocrinology and Metabolism* 88, 5232–5239.

Gleason, D. F. & Mellinger, G. T. (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *Journal of Urology* 111, 58–64.

de Gooyer, M. E., Oppers-Tiemissen, H. M., Leysen, D., Verheul, H. A. & Kloosterboer, H. J. (2003) Tibolone is not converted by human aromatase to 7-alpha-methyl-17-alpha-ethynylestradiol (7-alpha-MEE): analyses with sensitive bioassays for estrogens and androgens and with LC–MSMS. *Steroids* 68, 235–243.

Hernandez, J. & Thompson, I. M. (2004) Diagnosis and treatment of prostate cancer. *Medical Clinics of North America* 88, 267–279.

Israeli, R. S., Powell, C. T., Fair, W. R. & Heston, W. D. (1993) Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. *Cancer Research* 53, 227–230.

Kumar, N., Didolkar, A. K., Monder, C., Bardin, C. W. & Sundaram, K. (1992) The biological activity of 7-alpha-methyl-19-nortestosterone is not amplified in male reproductive tract as is that of testosterone. *Endocrinology* 130, 3677–3683.

Kumar, N., Suvisaari, J., Tsong, Y. Y., Aguillaume, C., Bardin, C. W., Lahteenmaki, P. & Sundaram, K. (1997) Pharmacokinetics of 7-alphamethyl-19-nortestosterone in men and cynomolgus monkeys. *Journal of Andrology* 18, 352–358.

Kumar, N., Crozat, A., Li, F., Catterall, J. F., Bardin, C. W. & Sundaram, K. (1999) 7-alpha-methyl-19-nortestosterone, a synthetic androgen with high potency: structure-activity comparisons with other androgens. *The Journal of Steroid Biochemistry and Molecular Biology* 71, 213–222.

LaMorte, A., Kumar, N., Bardin, C. W. & Sundaram, K. (1994) Aromatization of 7-alpha-methyl-19-nortestosterone by human placental microsomes *in vitro*. *The Journal of Steroid Biochemistry and Molecular Biology* 48, 297–304.

Lee, C., Sutkowski, D. M., Sensibar, J. A., Zelner, D., Kim, I., Amsel, I., Shaw, N., Prins, G. S. & Kozlowski, J. M. (1995) Regulation of proliferation and production of prostate-specific antigen in androgen-sensitive prostatic cancer cells, LNCaP, by dihydrotestosterone. *Endocrinology* 136, 796–803.

Luke, M. C. & Coffey, D. S. (1994) Human androgen receptor binding to the androgen response element of prostate specific antigen. *Journal of Andrology* 15, 41–51.

Moore, R. & Wilson, J. (1972) Localization of the reduced nicotinamide adenine dinucleotide phosphate: Δ^4-3-ketosteroid 5-alpha-oxireductase in the nuclear membrane of the rat ventral prostate. *Journal of Biological Chemistry* 247, 958–967.

Negro-Vilar, A. (1999) Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *Journal of Clinical Endocrinology and Metabolism* 84, 3459–3462.

Noe, G., Suvisaari, J., Martin, C., Moo-Young, A. J., Sundaram, K., Saleh, S. I., Quintero, E., Croxatto, H. B. & Lahteenmaki, P. (1999) Gonadotrophin and testosterone suppression by 7-alpha-methyl-19-nortestosterone acetate administered by subdermal implant to healthy men. *Human Reproduction* 14, 2200–2206.

Rittmaster, R. (1997) 5-alpha-reductase inhibitors. *Journal of Andrology* 18, 582–587.

Ryan, C. J. & Small, E. J. (2004) Advances in prostate cancer. *Current Opinion in Oncology* 16, 242–246.

Sanchez de la Muela, P., Povo Castilla, J., Bobillo de Lamo, M., Rosales, F. & Perez-Piqueras, J. L. (1995) Microparticle immunoenzymatic assay for detection of prostate specific antigen: characterization of the technique and comparative analysis with a monoclonal immunoradiometric assay. *Military Medicine* 160, 416–419.

Shao, T. C., Li, H. L., Kasper, S., Matusik, R., Ittmann, M. & Cunningham, G. R. (2006) Comparison of the growth-promoting effects of testosterone and 7-alpha-methyl-19-nortestosterone (MENT) on the prostate and levator ani muscle of LPB-tag transgenic mice. *Prostate* 66, 369–376.

Su, Z. Z., Lin, J., Shen, R., Fisher, P. E., Goldstein, N. I. & Fisher, P. B. (1996) Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen gene PCTA-1 a member of the galectin gene family. *Proceeding of the National Academy of Science U S A* 93, 7252–7257.

Sundaram, K., Kumar, N. & Bardin, C. W. (1993) 7-alpha-methyl-nortestosterone (MENT): the optimal androgen for male contraception. *Annals of Medicine* 25, 199–205.

Sundaram, K., Kumar, N. & Bardin, C. W. (1994) 7-alpha-methyl-19-nortestosterone: an ideal androgen for replacement therapy. *Recent Progress in Hormone Research* 49, 373–376.

Suvisaari, J., Moo-Young, A., Juhakoski, A., Elomaa, K., Saleh, S. I. & Lahteenmaki, P. (1999) Pharmacokinetics of 7-alpha-methyl-19-nortestosterone (MENT) delivery using subdermal implants in healthy men. *Contraception* 60, 299–303.

Tanaka, A., Furuya, A., Yamasaki, M., Hanai, N., Kuriki, K., Kamiakito, T., Kobayashi, Y., Yoshida, H., Koike, M. & Fukuyama, M. (1998) High frequency of fibroblast growth factor (FGF) 8 expression in clinical prostate cancers and breast tissues, inmunohistochemically demonstrated by a

newly established neutralizing monoclonal antibody against FGF8. *Cancer Research* 58, 2053–2056.

Tanaka, S., Uedo, H., Mafune, K., Mori, M., Wands, J. & Sugimachi, K. (2001) A novel isoform of human fibroblast growth factor 8 is induced by androgens and associated with progression of esophageal carcinoma. *Digestive Diseases and Sciences* 46, 1016–1021.

Walton, M., Kumar, N., Baird, D., Ludlow, H. & Anderson, R. (2007) 7-alpha-Methyl-19-Nortestosterone (MENT) vs testosterone in combination with etonogestrel implants for spermatogenic suppression in healthy men. *Journal of Andrology* 28, 679–688.

Wu, J., Payson, R., Lang, J. & Chiu, I. (1997) Activation of fibroblast growth factor 8 gene expression in human embryonic carcinoma cells. *Journal of Steroid Biochemistry and Molecular Biology* 62, 1–10.

Yamagata, K., Urakami, K., Ikeda, K., Ji, Y., Adachi, Y., Arai, H., Sasaki, H., Sato, K. & Nakashima, K. (2001) High expression of apolipoprotein E mRNA in the brains with sporadic Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders* 12, 57–62.

