Pygeum africanum extract inhibits proliferation of human cultured prostatic fibroblasts and myofibroblasts

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Accepted for publication 12 July 2006

OBJECTIVE

To investigate the effect of *Pygeum africanum* (*PA*) extract on the proliferation of cultured human prostatic myofibroblasts and fibroblasts; this extract is used for treating urinary disorders associated with benign prostatic hyperplasia (BPH).

MATERIALS AND METHODS

Primary cultures of prostatic stromal cells were obtained from histologically confirmed human BPH by enzymatic digestion. Cell proliferation was measured by 5-bromo2′-deoxy-uridine (BrdU) incorporation assays, and cytotoxicity by luminescent quantification of adenylate kinase activity.

RESULTS

Cultured cells were labelled by an anti-vimentin antibody, and most of them by an α–smooth-muscle-actin antibody, revealing the presence of fibroblasts and myofibroblasts. BrdU incorporation tests showed that proliferation of cultured human stromal cells, stimulated by fetal calf serum, by basic fibroblast growth factor and by epidermal growth factor, was dose-dependently inhibited by *PA* extract (5–100 μg/mL). Except at 100 μg/mL, no acute cytotoxicity of the extract was detected after 24 h of culture. Similarly, the extract dose-dependently inhibited the proliferation of Madin–Darby canine kidney epithelial cells, but to a lesser extent; whatever the dose of extract, no acute toxicity was evident on this cell line.

CONCLUSION

PA extract inhibits the proliferation of cultured human prostatic myofibroblasts and fibroblasts. We propose that cultured human prostatic cells offer a reliable model for preclinical screening of therapeutic agents, and to study the mechanisms underlying the inhibition of proliferation.

KEYWORDS

BPH, phytotherapy, *Pygeum africanum*, primary culture, prostatic myofibroblasts and fibroblasts, cell proliferation

INTRODUCTION

BPH and associated symptoms of urinary tract outlet obstruction appear to be a consequence of hyperproliferation, mainly within the stromal component, with increased tonicity of the prostatic smooth muscles. Nodular and fibromuscular hyperplasia are the major cellular events involved in BPH pathogenesis. An inflammatory process is also present within the prostate, which often shows an accumulation of neutrophils in the lumina and of mononuclear cells in the stroma [1]. Surgery is considered to be the most effective treatment, and is often used in men with severe symptoms. Drug treatments are also available, including *Pygeum africanum* (*PA*) extract (from the bark of the African plum tree, which contains flavonoids and β-sitosterol), which has been used for the past 25 years to treat micturition disorders associated with BPH. In animals, *PA* extract improves the contractility of detrusor muscle associated with changes in the expression of myosin isoforms [2–5], reduces BOO [6,7], and inhibits the inflammatory process [8]. Most of these results are presumably related, at least in part, to its action as an inhibitor of 5-lipooxygenase metabolite production [9].

For proliferation, growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), are crucial in prostatic stromal proliferation during BPH. The high levels of these factors in BPH, associated with extensive cell proliferation, suggest their involvement in the 'awakening' of mesenchymal tissue, leading to the formation of the primitive fibrostromal nodule and then to BPH [10–13]. Some studies showed an induction of cultured stromal cell proliferation by bFGF and EGF [14–17], and Paul-Braquet *et al.* [16] found that proliferation of prostatic fibroblasts from 3T3 mice stimulated by bFGF was inhibited by *PA* extract. Yablonsky *et al.* [17] obtained similar results on rat prostatic fibroblast proliferation stimulated by growth factors or by phorbol esters, suggesting that the extract could also inhibit protein kinase C (PKC)-driven cell proliferation. Finally, in the cancer cell line PZ-HPV-7, *PA* extract counteracted the mitogenic action of EGF and blocked the transition from the G1- to S-phase of the cell cycle [18]; in that study, the extract was described as exerting a potent antimitogenic action on epithelial cells from BPH explants. Together, these observations confirm an inhibitory effect of *PA* extract on cell proliferation.

These observations are from *in vitro* studies on cell cultures from animal prostates or on established cell lines. So far, there have been no studies of the effects of *PA* extract on cells from human BPH. In the present study, the effects of *PA* extract on proliferation and cytotoxicity were studied *in vitro* on myofibroblasts and fibroblasts from human prostates.

MATERIALS AND METHODS

From histologically confirmed human BPH, 14 specimens were obtained surgically in accordance with French Government regulations and local committee guidelines. The prostate tissue was cut into small pieces, which were incubated for 30 min at 37 °C under gassing (100% O_2), in 10 mL enzyme solution containing 0.1% collagenase P (4.04 U/mL, Roche Diagnostics, Mannheim, Germany), 0.03% pronase from *Streptomyces griseus* and 0.08% soybean trypsin inhibitor (Sigma, St. Louis, MO, USA), dissolved in sterile modified Ringer buffer (132 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 2.2 mM $MgSO_4$, 10 μM $CaCl_2$, 0.02% phenol red, 11 mM glucose, 25 mM Hepes-Na +, 0.2% BSA, 100 000 U/L penicillin, 100 000 U/L streptomycin, 100 mg/L gentamicin, 0.5 mg/L cefotaxim, pH 7.4). After three washes with buffer, the remaining tissue was incubated in the same conditions for 30 min, then filtered through a 500 μm-grid nylon mesh, centrifuged (150 ***g***, 5 min, 20 °C) and finally washed in fresh sterile buffer (150 ***g***, 5 min, 20 °C). Viability was determined by the trypan-blue exclusion method.

For cell culture, all subsequent operations were done aseptically. After enzymatic dissociation, freshly isolated cells were re-suspended in complete culture medium: MCDB 131 supplemented with 1 × L-glutamine; (Cambrex Bio Science, Rokland, ME, USA), 5% fetal calf serum (FCS, Eurobio, Les Ulis, France), 1 × MEM vitamins solution, 1 × Insulin-Tranferrin-Selenium Liquid Media Supplement, and 1% (v/v) antimycotic/antibiotic solution (all Sigma). Cells were then centrifuged (100 ***g***, 20 s, 20 °C) and the supernatant, containing isolated prostatic cells, was seeded in dishes coated with mouse collagen IV (500 000 cells for a 60-mm dish; Becton Dickinson, Bedford, MA, USA). After 4 h, dishes were washed with sterile PBS and fresh culture medium added; the culture medium was renewed every 2–3 days. When the cultured cells reached confluence, they were washed twice with sterile PBS and detached with trypsin/EDTA solution (Cambrex). The cells were centrifuged (150 ***g***, 5 min, 20 °C) and split at a ratio of 1 : 3. Cell strains could be kept for at least six or seven passages, or conserved at −80 °C in 90% FCS-10% dimethylsulphoxide.

The Madin–Darby canine kidney epithelial cell line (MDCK cells) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1 × L-glutamine, 5% FCS and 1% (v/v) antibiotic solution (Sigma).

For immunocytochemistry, the cultured cells were fixed with 3.7% paraformaldehyde for 5 min. After three washes with PBS, cells were permeabilized with 0.2% (v/v) Triton X-100 in PBS. The cells were then incubated with the appropriate primary antibodies for 1 h at room temperature: anticytokeratin (PAN, final dilution 1 : 50, Zymed Laboratories, San Francisco, CA, USA), anti-smooth muscle myosin heavy chain (clone HSM-V, final dilution 1 : 200, Sigma), anti-α-smooth muscle actin (clone 1A4, final dilution 1 : 200, Sigma), antivimentin (H-84 polyclonal, final dilution 1 : 1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and antidesmin (clone DE-U-10, final dilution 1 : 20, Sigma). The labelled proteins were detected using rhodamine-conjugated anti-monoclonal antibody or Fluor-conjugated anti-polyclonal antibody. The cells were then incubated with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, 5 μg/mL, Molecular Probes Inc., Eugene, OR, USA) in PBS and observed using a LEICA DMR microscope coupled with a Coolsnap fx Photometris Camera.

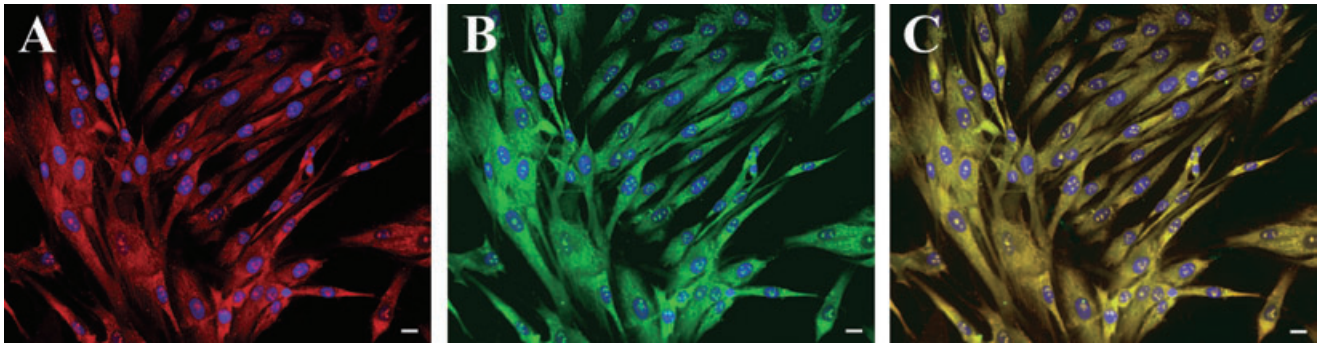
The *PA* extract, obtained after solvent extraction of *PA* bark, was the property of Laboratories Fournier-Pharma (Garches, France). All samples of extract were batch-to-batch standardized (one of the methods used to for this standardization was the assay of β-sitosterol contents). A sterile stock solution of 20 mg/mL was prepared in 100% ethanol and stored at −20 °C. This ethanol solution accounts for ≈80% of the *PA* extract. The bFGF and EGF sterile stock solutions were prepared at 10 μg/mL PBS. All subsequent dilutions were in 0.5% ethanol (final concentration).

For the proliferation assay, cells were derived from 14 patients. Human prostatic myofibroblasts and fibroblasts (HPMF) from the second to fifth passages, and MDCK cells, were used; each passage was tested several times. HPMF were seeded on 96-well plates coated with mouse collagen IV (1000 cells/dish; Becton Dickinson) in complete culture medium. MDKC cells were seeded on 96-well plates (1000 cells/dish), in complete DMEM. After 48 h, cells were washed twice with PBS and deprived in MCDB 131 supplemented with 1 × L-glutamine and 0.25% FCS (minimum FCS percentage to assure cell survival) or in serum-free DMEM, respectively. After 24 h, cells were incubated in 0.25% MCBD or in serum-free DMEM containing bFGF, EGF or FCS with or with no *PA* extract, and 10 μM 5-bromo-2′-deoxyuridine (BrdU), for a further 24 h (0.5% ethanol, final concentration). The cells were then fixed and permeabilized with pre-cooled ethanol/HCl (70% ethanol, 0.5 M HCl) for 30 min at −20 °C. Proliferation tests used the BrdU Labeling and Detection Kit III (Roche Diagnostics, Mannheim, Germany), according to manufacturer's instructions. BrdU incorporation was measured using a microtitre-plate reader at 405 nm, with a reference wavelength at 490 nm. A non-specific control level from cells with no BrdU was subtracted from each value, which was then referred to its corresponding negative control.

Cytotoxicity assays used the Toxilight™ kit (Cambrex) according to the manufacturer's instructions; this nondestructive bioluminescent assay measures the release, in the culture medium, from damaged cells of adenylate kinase (AK; an enzyme from the intermembrane space of mitochondria). By a two-step reaction involving ATP and luciferin, the emitted light-intensity is linearly related to AK concentration. For this, 10 μL of the culture medium of each condition was put on a white plate adapted for luminescence detection in a β-counter. The detection medium was added to each well and the plate incubated for 5 min. Luminescence was measured in a counter (WALLA JET 1450) and analysed with Microbeta Workstation software.

Four non-specific controls were used for each incubation condition: MCDB 131 supplemented with (i) 0.25% FCS; (ii) 0.25% FCS and 5% FCS; (iii) 0.25% FCS and 25 ng/mL EGF, and (iv) 0.25% FCS and 25 ng/mL bFGF. These values were subtracted from the values for each corresponding condition, and each value was then referred to its own negative control (in the absence of *PA* extract). The positive control represents the maximum AK activity released by complete cell lysis, obtained with Toxilight™ 100% Lysis Reagent Set (Cambrex). The efficacy of extract treatment was evaluated by one-way ANOVA followed by a mean comparison (*t*-test) of treated and untreated cells.

FIG. 1. Immunocytochemical staining of cytoskeletal markers in cultured human prostate cells: A, anti-α-smooth muscle actin labelled in red; B, anti-vimentin in green. C, the merged images from A and B. Nuclei were labelled in blue with DAPI (bar, 10 μm).



RESULTS

CHARACTERIZATION OF HUMAN PROSTATIC MYOFIBROBLASTS AND FIBROBLASTS (HPMF) IN CULTURE

Dissociation of the prostate tissue produced a heterogeneous cell population of stromal cells (fibroblasts, myofibroblasts and smooth muscle cells) and epithelial cells. Cell viability after isolation was >88%, as determined by the trypan-blue exclusion method. Freshly isolated cells were then grown on plates pre-coated with an extracellular matrix component. Various tests allowed us to establish that stromal cells had better adherence on mice collagen IV matrix than on laminin or on fibronectin (data not shown).

After every passage, to identify the different cell types present in the population, immunofluorescent detection experiments were done with antibodies against smooth muscle or epithelial differentiation markers (cytokeratins, α-actin, myosin, desmin, and vimentin). As expected for such proliferating cells, we detected a rapid disappearance of the late smooth-muscle differentiation marker desmin, followed by that of myosin expression, and by a decrease in the number of cells labelled with anti-α-actin (Table 1). However, although there were fewer cells expressing α-actin, this marker never disappeared completely, suggesting the coexistence of fibroblasts and myofibroblasts (Fig. 1). This was corroborated by an increase in anti-vimentin labelling.

Finally, the primary culture conditions favoured the selection of stromal cells and increased their enrichment, as there was

Passage	Vimentin	α-actin	Myosin	Desmin	Cytokeratin
0	++	++	++	+	+/–
1	+++	++	+	+/–	–
2	+++	++	+/–	–	–
3	+++	++	-	–	–
4	+++	+	-	–	–
5	+++	+	-	–	–
6	+++	+	-	–	–

TABLE 1 Characterization of the cultured cells at various passages. Intensity of staining: +, positive; +/–, few cells; –, negative

*FIG. 2. DNA synthesis (BrdU incorporation) in human prostate cells in the presence of growth factor. Data are the mean (SEM) proliferation index, which represents the ratio of BrdU incorporation in the presence of growth factor (at different concentrations) or 5% FCS vs in the absence of growth factor (control cells). **P < 0.01; ***P < 0.001.*

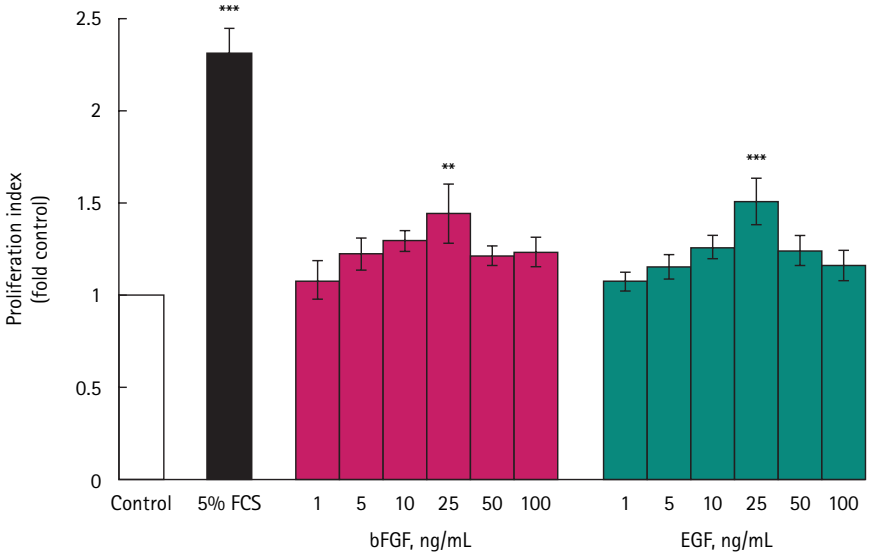
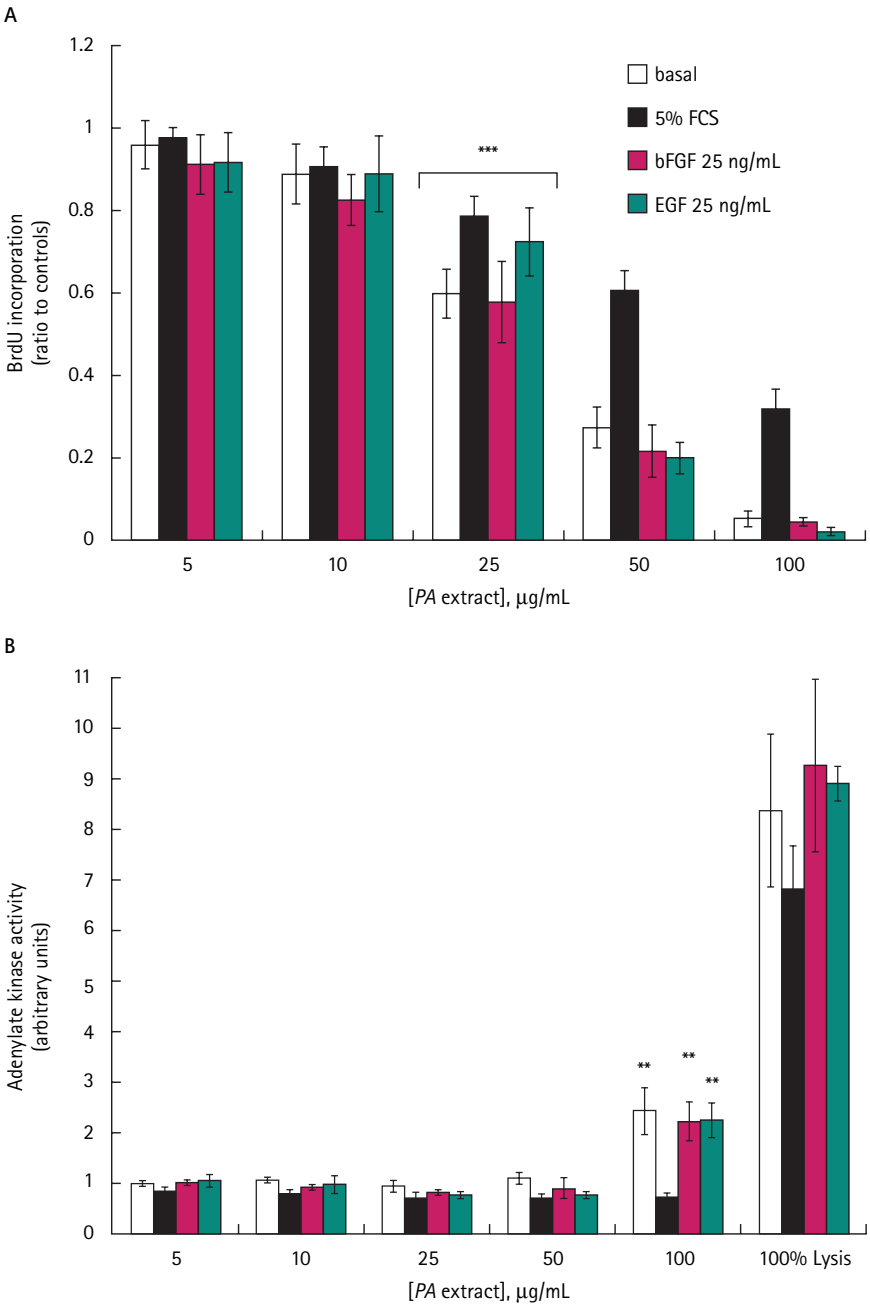


FIG. 3. ***A****,* PA *extract-induced inhibition of BrdU incorporation in human prostate cells stimulated or not by growth factor. Data are the mean (SEM;* n = 12*). For each culture condition, the values are the ratio of BrdU incorporation in the presence vs the absence of extract. Basal, 5% FCS; bFGF: (25 ng/mL); EGF (25 ng/mL): cells grown, respectively, in MCDB 131/0.25% FCS, MCDB 131 plus 5% FCS, MCDB 131 plus 25 ng/mL bFGF, MCDB 131 plus 25 ng/mL bFGF.* ***B****, Cytotoxicity assays: bioluminescence resulting from AK activity in the culture medium of prostate cells, stimulated or not by growth factor, in the presence of extract. Each value was referred to that measured in the absence of extract (mean (SEM),* n = 10*). FCS 5%; bFGF 25 ng/mL; EGF 25 ng/mL. Statistical significance is given in Table 2.*



no labelling with anti-cytokeratin after passaging the cell once, reflecting the absence of epithelial cells in the cultures.

EFFECT OF GROWTH FACTORS ON CELL GROWTH

BrdU incorporation was used to evaluate the least amount of FCS needed to be added to MCDB 131 to maintain HPMF survival during deprivation. Because of the heterogeneity of the BPH samples, the minimum amount of FCS needed was 0.1–0.25% (data not shown). To standardize experiments, all further experiments used cells grown in MCDB 131 supplemented with 0.25% FCS.

HPMF were grown in medium supplemented with 0.25% FCS for 24 h and for a further 24 h in 5% FCS, or with the indicated concentration of bFGF or EGF. Proliferation was then determined by BrdU incorporation assay. With 5% FCS, there was a 2.3-fold increase in BrdU incorporation ($P < 0.001$, $n = 27$ Fig. 2). Both bFGF and EGF had a maximum proliferating effect at 25 ng/mL. This effect was lower than with FCS but was significant (bFGF: 1.4-fold, $P < 0.01$, $n = 16$; EGF: 1.5-fold, $P < 0.001$, $n = 12$).

EFFECTS OF PYGEUM AFRICANUM (PA) EXTRACT ON HPMF

HPMF were incubated with 5–100 µg/mL *PA* extract in MCDB 131/0.25% FCS, supplemented or not with 5% FCS, 25 ng/mL bFGF or 25 ng/mL EGF during 24 h. The extract reduced BrdU incorporation dose-dependently in all culture conditions (MCDB 131/0.25% FCS, 5% FCS, 25 ng/mL bFGF, or 25 ng/mL EGF; Fig. 3A). There was a significant difference in BrdU incorporation in the presence of *PA* extract compared to the controls (in the absence of extract) for 25, 50 and 100 µg/mL of extract (Table 2). The *PA* extract inhibited cells grown in 5% FCS less than cells grown in the presence of growth factors. This effect was also significantly different from 25 µg/mL (Table 2).

Cytotoxicity was evaluated by measuring mitochondrial AK activity, released from damaged cells in the culture medium. No toxic effect was detected for extract concentrations of <100 µg/mL, whatever the culture conditions (Fig. 3B). At 100 µg/mL, the extract had a cytotoxic effect, significantly different from controls (same culture conditions with

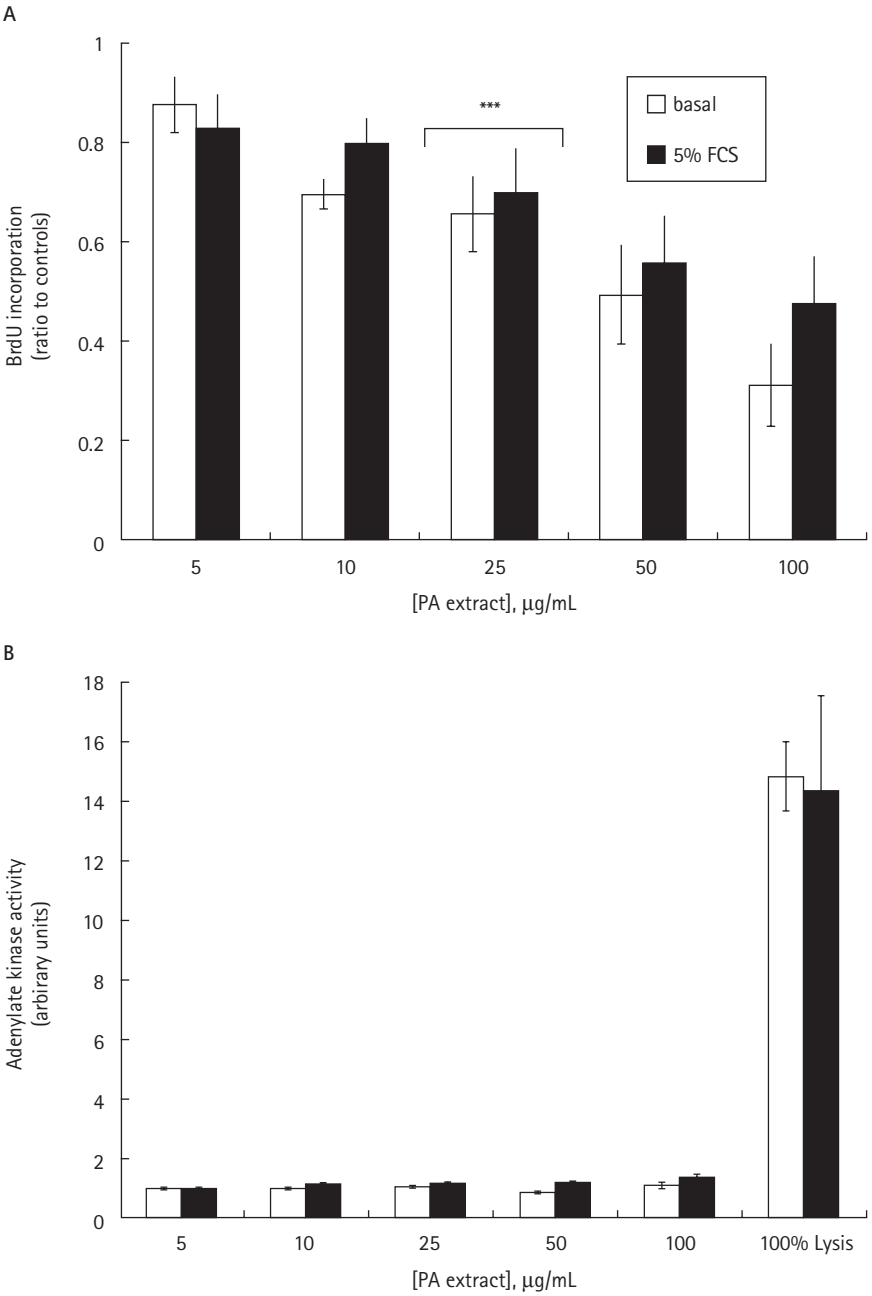
no extract, for cells grown in medium supplemented by growth factor; $P < 0.01$, $n = 12$, Table 2). However, compared with total cell lysis, which represents the maximum AK activity (maximal toxicity), the cytotoxic effect of 100 μg/mL extract was three to four times less. At 100 μg/mL the extract had no toxic effect on cells grown in 5% FCS, suggesting a protective effect of FCS.

EFFECTS OF PA EXTRACT ON MDCK CELLS

To evaluate whether *PA* extract had a selective effect on HPMF, we did similar experiments on MDCK cells incubated in DMEM supplemented or not with 5% FCS for 24 h in the presence of extract (5–100 μg/mL). The extract dose-dependently inhibited BrdU incorporation into cells grown in serum-free DMEM or supplemented by 5% FCS (Fig. 4A). BrdU incorporation in the presence of extract was significantly different from controls (in the absence of extract) for 25, 50 and 100 μg/mL extract (Table 2). However, the inhibition of proliferation was less for MDCK cells than for HPMF (Figs 3A, 4A); at 50 μg/mL extract, inhibition was 0.27 (0.05) (73% inhibition) for HPMF and 0.49 (0.09) (51% inhibition) for MDCK cells ($P < 0.001$).

For MDCK cells, all values of AK activity in the presence of *PA* extract were not significantly different from controls (in the absence of extract; Table 2). There was no toxicity at any extract concentration used (5–100 μg/mL; Fig. 4B).

FIG. 4. ***A***, PA *extract-induced inhibition of BrdU incorporation in MDCK cells stimulated or not by 5% FCS. Data are the mean (SEM),* n = *12. For each culture condition, the values are the ratio of BrdU incorporation in the presence vs the absence of extract. Basal, 5% FCS; bFGF 25 ng/mL; EGF 25 ng/mL: cells grown, respectively, in MCDB 131/0.25% FCS, MCDB 131 plus 5% FCS, MCDB 131 plus 25 ng/mL bFGF, MCDB 131 plus 25 ng/mL FGF.* ***B***, *Cytotoxicity assays: bioluminescence resulting from AK activity in the culture medium of MDCK cells, stimulated or not by 5% FCS, in various extract concentrations. Each value was referred to that measured in the absence of extract (mean (SEM),* n = *10). FCS 5%; bFGF 25 ng/mL; EGF 25 ng/mL. Statistical significance is given in Table 2.*



DISCUSSION

BPH and BOO involve hyperproliferation within the stromal component, associated with increased tonicity of prostatic smooth muscles. As well as surgical approaches, drug treatments such as *PA* extract have been used for 25 years to treat micturition disorders associated with BPH. The direct effects of the extract on the inhibition of stromal cell proliferation were studied *in vitro*, but only on cell lines or cultured cells from animals. In the present study, we assessed the effect of *PA* extract on the proliferation of prostatic myofibroblasts and fibroblasts in culture from human BPH.

The cellular model was established and validated by immunocytochemistry. A large majority of cells expressed α-actin, but lost expression of markers of late smooth muscle differentiation, such as myosin or desmin. This, together with the increased expression of vimentin, confirmed the presence of fibroblasts and myofibroblasts in the cell population. Hepatic and renal fibrosis are influenced by many factors (noradrenergic innervation, steroid, growth factors, etc.), which can induce cell differentiation into

TABLE 2 P *values for statistical comparisons between* PA *extract-treated and untreated HPMF and MDCK cells in proliferation and cytotoxicity tests. The efficacy of the extract on cultured cells was evaluated using one-way* ANOVA *followed by a mean comparison (t-test) between treated and untreated HPMF and MDCK cells.*

Dose PA extract, μg/mL	Medium	5% FCS	25 ng/mL EGF	25 ng/mL bFGF
HMPF				
Proliferation				
ANOVA F, P	38.71, <0.001	23.71, <0.001	22.06, <0.001	25.1, <0.001
5	0.627	(–) 0.065	0.811	0.573
10	0.647	0.432	0.533	0.050
25	<0.001	0.012	0.018	<0.001
50	<0.001	<0.001	<0.001	<0.001
100	<0.001	<0.001	<0.001	<0.001
Toxicity				
ANOVA F, P	11.37, <0.001	1.53, 0.190	23.45, <0.001	18.02, <0.001
5	0.940	0.680	0.834	0.680
10	0.627	0.351	0.767	0.272
25	0.865	0.066	0.213	0.073
50	0.414	0.009	0.107	0.091
100	<0.001	<0.001	<0.001	<0.001
MDCK,				
Proliferation				
ANOVA F, P	13.85, <0.001	13.07, <0.001		
5	0.935	0.035		
10	0.052	0.039		
25	<0.001	0.001		
50	<0.001	0.007		
100	<0.001	<0.001		
Toxicity				
ANOVA F, P	3.41, 0.006	2.11, 0.067		
5	0.636	0.033		
10	0.895	0.035		
25	0.331	0.190		
50	0.271	0.139		
100	0.023	0.009		

myofibroblasts [19,20]. Myofibroblasts, being major producers of extracellular matrix, are thought to contribute directly to liver fibrosis [20–23]. If a similar mechanism occurs in BPH, our model might be valuable for the study of antiproliferation agents in this disease. However, the major difficulty came from the variability of BPH between human prostates. Survival tests revealed that the minimum amount of FCS to assure cell viability was not the same for each culture.

PA has many components: phytosterols, pentacyclic triterpenes, ferulic acid esters, etc. Each has its own mode of action (e.g. interaction with specific receptor or activation/inhibition of intracellular enzymes in specific pathways), the consequence of which is modulation of bladder/prostate contractility [2–5] and an anti-inflammatory action [8,24]. Thus it is difficult to study a specific mode of action for each component, and more important to evaluate the overall effects of the extract.

The lowest concentration of *PA* extract that had a significant inhibitory effect on cells stimulated or not by FCS and EGF or bFGF was 25 μg/mL. Similar results were reported for the fibroblast 3T3 cell line [16] and rat fibroblasts [17]. We sought a cytotoxic effect of *PA* extract on HPMF by measuring mitochondrial AK activity in the culture medium. This technique, which gives the amount of dead cells and not of living cells, led us to conclude that *PA* extract did not have acute cell toxicity at <100 μg/mL. However, when cells were cultured in the presence of 5% FCS, there was no cell toxicity even at 100 μg/mL, suggesting a protective effect of FCS.

Finally, *PA* extract had differential effects on HPMF and MDCK cells. At 100 μg/mL, the extract had a cytotoxic effect on unstimulated HPMF cells, but not on unstimulated MDCK cells. Moreover, at 50 μg/mL, the extract caused significantly less inhibition of proliferation on MDCK cells than on HPMF (73% vs 51%). Similar experiments on other cell types would therefore be interesting.

PA extract had a growth inhibitory effect on HPMF. Its mode of action is unknown. In a previous paper, this growth inhibitory effect of the extract on rat fibroblasts was detected on cell proliferation caused by PKC-specific activators (12-O tetradecanoyl phorbol-13-acetate, and phorbol-12,13-dibutyrate) [17]. The authors suggested that *PA* extract caused the growth inhibitory effect, at least in part, through the PKC pathway, but in a different way to that of staurosporin, which results in an irreversible and specific inhibition of PKC.

Another study suggested a regulatory role of *PA* extract on prostatic components of the adenylate cyclase signalling pathway; the extract caused an increase in the efficacy of vaso-intestinal peptide on adenylate cyclase stimulation, without modulating vaso-intestinal peptide binding to plasma membranes [25]. This effect could be mediated through: (i) an increase of vaso-intestinal peptide-induced adenylate cyclase stimulation through α(s) G-subunit; (ii) α(i) activation by GTP analogue Gpp[NH]p at low concentrations (in the presence of forskolin); (iii) over-expression of α(s), α(i1/2), and α(i3/0) subunits.

Finally, a recent study showed that *PA* extract co-treatment (or post-treatment) with dihydrotestosterone in rats suppressed the effects of dihydrotestosterone on micturition, and that co-treatment with the extract reduced the developing increase in prostatic weight, whereas *PA* extract after treatment did not reduce the pre-existing condition of an hormonally enlarged prostate [7]. The primary role of androgens on proliferation and maintenance of prostatic cell functions is well documented [26–28]. Therefore, 'cross-talk' between androgen, PKC and adenylate cyclase pathways might be involved in the effect of the *PA* extract. Further studies will concentrate on the effects of the extract on steroid-stimulated HPMF, to obtain more information about the mechanism involved in inhibition of HPMF proliferation.

Clinical studies, including a European double-blind placebo-controlled study, showed that *PA* extract provided a significant improvement in the combined outcome of urological symptoms and flow measures [29,30]. In the present study, treatment of cells with *PA* extract inhibited cell proliferation, induced or not by growth factors, at 25 μg/mL, where no cytotoxicity was apparent. The present results suggest that the clinical effects of *PA* extract could be due to inhibition of proliferation of HPMF. Further studies are in progress of the effect of *PA* extract on newly highlighted prostatic factors (like IGF II or Cyr 61), and on stimulation of inflammation by prostaglandins [31–35].

Finally, even if the proliferation rate of our cultured cells is faster than in human BPH, cultured myofibroblasts and fibroblasts from human BPH would be suitable to study *in vitro* the intracellular mode of action of pharmacological agents on cell proliferation and differentiation. The present work shows that cultured HPMF from primary cultured stromal cells could offer a reliable model for preclinical screening of therapeutic agents, and for studying the underlying molecular mechanisms. Further experiments are needed to specify the intracellular inhibitory pathway triggered by *PA* extract.

CONFLICT OF INTEREST

P. Costa is a paid consultant to sponsor; M. Haddoum is an employee of sponsor. Source of funding: governmental grants and Fournier-Pharma grants.

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Abbreviations: ***PA***, *Pygeum africanum*; **bFGF**, basic fibroblast growth factor; **EGF**, epidermal growth factor; **PKC**, protein kinase C; **AK**, adenylate kinase, **FCS**, fetal calf serum; **BrdU**, 5-Bromo-2′-deoxy-uridine; **DAPI**, 4′,6-diamidino-2-phenylindole; **DMEM**, Dulbecco's Modified Eagle Medium; **HPMF**, human prostatic myofibroblasts and fibroblasts; **MDCK**, Madin–Darby canine kidney (epithelial cell line).