Research Paper

Eur Urol 1992;21:309–314

F. Di Silverio[a]
G. D'Eramo[a]
C. Lubrano[b]
G.P. Flammia[a]
A. Sciarra[a]
E. Palma[b]
M. Caponera[a]
F. Sciarra[b]

[a] Department of Urology 'U. Bracci' and
[b] Institute of Clinical Medicine V, University of Rome 'La Sapienza', Rome, Italy

Evidence that *Serenoa repens* Extract Displays an Antiestrogenic Activity in Prostatic Tissue of Benign Prostatic Hypertrophy Patients

Key Words

Benign prostatic hypertrophy
Antiestrogen
Serenoa repens
Estrogenic receptors

Abstract

A double-blind placebo-controlled study was performed in 35 benign prostatic hypertrophy (BPH) patients never treated before. The patients were randomized into two groups, the 1st (18 cases) receiving *Serenoa repens* extract (160 mg t.d.) for 3 months up to the day before the operation of transvesical adenomectomy and the 2nd (17 cases) receiving placebo. Steroid receptors were evaluated in the nuclear (n) and cytosolic (c) fraction using the saturation analysis technique (Scatchard analysis or single saturating-dose assay) for androgen (AR) and estrogen (ER) receptors and the enzyme immunoassay (EIA) for ER and progesterone receptors (PgR). Scatchard analysis of ERc and ERn revealed the presence of two classes of binding sites, one with high-affinity low-capacity binding and the other with low-affinity high-capacity binding. In the untreated BPH group, ER were higher in the n than in the c fraction: ERn were positive in 14 cases and ERc in 12 of 17 cases. In the BPH group treated with *S. repens* extract on the contrary, ERn were negative for both binding classes in 17 cases and ERc in 6 of 18 cases. Using EIA, ERn and ERc were detected in all 15 samples examined, but in the treated group, ERn were significantly ($p < 0.01$) lower than in the untreated group, whilst ERc remained almost unchanged. Similar results were obtained measuring PgR; the n fraction of the treated group prostatic samples was significantly ($p < 0.01$) lower than that of the untreated group. Finally, the determination of AR showed that ARn were positive in 6 of 10 untreated cases and in only 1 of 10 treated cases, whilst ARc were almost the same in the two groups. In conclusion, these findings show that *S. repens* extract is able to inhibit the nuclear estrogen receptors in prostatic tissue samples of BPH patients. The results obtained with the Scatchard analysis or the single saturating-dose assay are confirmed by ER-EIA and by PgR-EIA representing a marker of the estrogenic activity. A possible explanation for these findings is that *S. repens* extract contains at least two fractions, one with antiandrogenic, the other with antiestrogenic effect, able to block the translocation of ERc to the nuclei by competition. It cannot be excluded, however, that the primary effect is antiestrogenic and that the inactivation of AR and PgR is secondary to ER blockade.

Dr. Franco di Silverio
Dipartimento di Urologia U. Bracci
Università degli Studi di Roma La Sapienza
Policlinico Umberto I, Viale del Policlinico
I-00161 Rome (Italy)

© 1992 S. Karger AG, Basel
0302–2838/92/0214–0309
$2.75/0

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM

Introduction

The lipidosterolic *Serenoa repens* extract of the dwarf palm tree used in the medical treatment of benign prostatic hyperplasia (BPH) is a multisite inhibitor of androgen action competing with DHT at androgen receptor (AR) level and affecting testosterone metabolism [1–4].

In vitro experiments on the rat ventral prostate, in fact, showed that *S. repens* extract inhibits the binding of ^{3}H-methyltrienolone and mibolerone, highly specific for AR at low concentrations, whilst other plant extracts tested were inactive [2–5].

Similar results were obtained in cultured genital skin fibroblasts, where *S. repens* extract displays about 70% of ^{3}H-DHT binding inhibition at nuclear receptor level and 90% inhibition at cytosolic receptor level [5].

This extract has also an inhibitory effect on the 5α-reductase in the rat ventral prostate, with a 50% reduction in testosterone conversion into DHT at a concentration of 100 μg/ml (progesterone has an IC_{50} value of 60 n*M*) and in foreskin fibroblasts, where DHT formation is decreased by 90% at a concentration of 5.7 U/ml [3, 5].

Furthermore 3α-oxidoreductase activity is also partially inhibited, with decreased formation of 5α-androstane-3α,17β-diol, in cultured foreskin fibroblasts obtained from normal infants or adults [5].

The possibility that *S. repens* extract acts at another steroid receptor level is not demonstrated. The present investigation is the first evidence that this plant extract is able to inhibit in prostatic tissue of BPH patients the estrogen receptors (ER).

Materials and Methods

Chemicals

(2,4,6,7-^{3}H)-estradiol-17β (SA 87–102 Ci/mmol) was obtained from New England Nuclear (Du Pont de Nemours, France) and stored for not more than 6 months at 0–4 °C. Diethylstilbestrol (DES), bovine serum albumine (BSA) and DNA were purchased from Sigma (St. Louis, Mo., USA). All other reagents were of analytical grade.

TEGM Buffer

Tris-HCl 0.01 *M*, EDTA 0.001 *M*, mercaptoethanol 0.002 *M*, sodium molybdate 0.02 *M*, PMSF 0.001 *M*, dithiothreytol 0.001 *M*, glycerol 10%, pH 7.4, at 25 °C. ER-enzyme immunoassay (EIA) and progesterone receptor (PgR)-EIA kits for estradiol and PgR were purchased from Abbott Laboratories (Chicago, Ill., USA).

Tissue Preparation

Prostatic tissue specimens, removed surgically by transvesical resection, were immediately processed or stored at – 70 °C. All samples were examined histologically and in each case BPH was confirmed. About 1 g of tissue was pulverized using a porcelain mortar in liquid nitrogen and homogenized in 4 vol of TEGM buffer. The homogenate was filtered through a double layer of organza and centrifuged at 800 *g* for 10 min at 4 °C to obtain cytoplasmic and nuclear fractions by washing and resuspending the pellets in the same buffer.

The cytoplasmic fraction was then centrifuged at 100,000 *g* for 60 min at 4 °C to obtain the cytosolic fraction, whilst the nuclear fraction was resuspended in TEGM buffer containing sodium molybdate 0.2 *M* and incubated for 30 min at 0–4 °C, then centrifuged at 100,000 *g* for 60 min to obtain the nuclear extract. Protein concentration was measured according to Bradford's [6] method and that of DNA according to Burton's [7] procedure.

ER Determination by Saturation Analysis (SA) in the Cytosol and Nuclear Extracts

The exchange of occupied receptors was obtained with overnight incubation at 0 °C in the presence of sodium thyocianate (0.5 *M*) for cytosol ER (ERc). The exchange of nuclear ER (ERn) was achieved with overnight incubation at 0 °C in the presence of sodium molybdate (0.2 *M*). The radioactive ligand used was tritiated estradiol, with or without 200- or 1,500-fold excess of DES for ERc and ERn, respectively. Free and bound ligands were separated by means of dextran-coated charcoal suspension (dextran 0.05, charcoal 0.5%). Aliquots of the supernatant were added to 4 ml Picofluor 30 and radioactivity measured in a Packard 460 CD scintillation spectrometer. Scatchard plot analyses were made with an interfaced IBM computer system mod. 5150. When a limited amount of tissue was available, ER levels were determined at a single ligand concentration of 10 n*M* for ERn and 5 n*M* for ERc.

AR were measured by a single saturating-dose assay in the cytosol and nuclear extract, after addition of sodium molybdate (0.2 *M*) to the nuclear suspension and centrifugation at 100,000 *g* for 60 min at 2 °C. Samples were incubated overnight at 0 °C in the presence of ^{3}H-R1881 (10 n*M*) and triamcinolone acetonide (5 μ*M*) with and without the addition of 200- or 1,500-fold excess of cold R1881 for cytosolic (ARc) and nuclear AR (ARn), respectively. The separation of free and bound ligands was achieved by means of dextran-coated charcoal suspension.

ER and PgR Determination by EIA in the Cytosol and Nuclear Extract

This assay was performed following the instructions provided by the Abbott Laboratories. In brief, aliquots of cytosol and nuclear extracts in duplicate were incubated with polystyrene beads coated with anti-ER or anti-PgR monoclonal anitbodies for 18 h at 2–8 °C. After washing, the beads were incubated with a 2nd monoclonal antibody preparation conjugated with horseradish peroxidase. Excess conjugate was removed and the beads were incubated in a *o*-phenylenediamine substrate solution for 30 min at room temperature for color development. Absorbance at 492 n*M* within 2 h for standard, control and specimens was read by spectrophotometer.

Patients

Investigations were carried out on 35 BPH patients aged 61–73 years, never treated before, in good general condition, presenting obstructive urinary symptoms from 1–3 years. All cases were submitted to rectal exploration, suprapubic and transrectal ultrasonography and to pressure-flow urine measurement.

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM

Table 1. ERn and ERc evaluated in BPH tissue samples by Scatchard analysis in patients untreated and treated with *S repens* extract

Scatchard analysis	ERn		ERc	
	untreated	treated	untreated	treated
Positivity, n (%)	6/7 (86)	1/8 (12.5)	5/7 (71)	6/8 (75)
High-affinity site fmol/mg DNA	6.183 ±3.761	0.8	0.640 ±0.592	0.493 ±0.321
Low-affinity site fmol/mg DNA	105.937 ±86.488	6.4	7.917 ±6.426	4.632 ±2.958

Table 2. AR and ER in the cytosolic and nuclear fraction evaluated by a single-point assay in 10 untreated BPH patients and in 10 treated with *S. repens* extract

	ARc fmol/mg protein	ARn fmol/mg DNA	ERc fmol/mg protein	ERn fmol/mg DNA
Positivity	9/10	6/10	7/10	8/10
Untreated	23.878 ±10.384	153.183 ±76.909	17.371 ±10.102	261.90 ±135.42
Positivity	6/10	1/10	6/10	0/10
Treated	24.467 ±12.697	108	17.533 ±11.815	ND

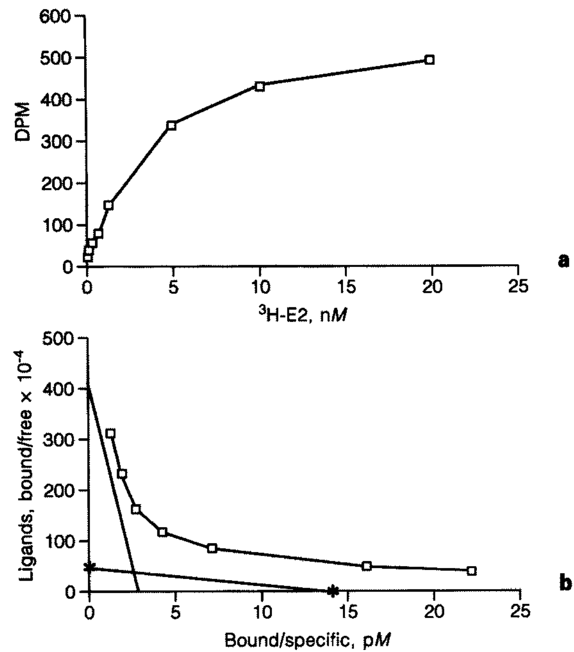

Fig. 1. Saturation curve (**a**) and Scatchard analysis (**b**) of ER in the nuclear extract of human BPH. Incubations were performed with 0.07–20 n*M* [^{3}H]17β-estradiol (^{3}H-E2) ± unlabeled DES (1,500-fold excess) for 24 h at 0 °C. Saturation data were corrected for nonspecific binding and plotted according to Scatchard. DPM = Disintegration per minute.

This was a double-blind placebo-controlled study in which the patients were randomized into two groups, the 1st consisting of 18 cases receiving *S. repens* extract (160 mg t.d.) for 3 months up to the day before the operation, the 2nd of 17 cases receiving placebo. All cases were submitted to transvesical adenomectomy using a cold blade.

Results

Saturation Analysis

Scatchard analysis of ERc and ERn revealed the presence of multiple estrogen binding sites, one class with high-affinity low-capacity binding (K_{d1} = 0.070 ± 0.038 SD in the nuclear fraction and 0.167 ± 0.116 SD in the cytosolic fraction) and the other with low-affinity high-capacity binding (K_{d2} = 5.910 ± 2.923 SD in the nuclear and 5.600 ± 3.011 SD in the cytosolic fraction; (fig. 1). The saturating dose of ^{3}H-17β-estradiol was equivalent to 5 n*M* in the cytosolic compartment and to 10 n*M* in the nuclear compartment.

ER concentrations in prostatic tissue of untreated BPH patients were significantly higher ($p < 0.001$) in the nuclear than in the cytosolic fraction for both binding classes, and ERn were positive in 6 of 7 cases. On the contrary, in treated patients, ERn were negative in 7 of 8 cases, the only positive case presenting very low receptor levels for both binding classes (table 1).

ERc concentrations in prostatic tissue of untreated BPH patients were positive in 5 of 7 cases, whilst in patients treated with *S. repens* extract they were positive in 6 of 8 cases. The differences for both high- and low-affinity binding classes between treated and untreated patients were not significant (table 1).

Using the single saturating-dose assay, ERc were positive in 7 of 10 untreated cases and ERn in 8. In the group treated with *S. repens* extract, ERc were positive in 6 of 10 cases, at concentrations not significantly different from the control group, whereas ERn were negative in all cases (table 2).

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM

Table 3. ERn, ERc and PgRn, PgRc evaluated in BPH tissue samples by EIA in patients untreated and treated with *S. repens* extract

EIA	Untreated	Treated
Positivity, n (%)	7/7 (100)	8/8 (100)
ERn, fmol/mg DNA	28.070 ± 10.071	15.145 ± 8.564
PRn, fmol/mg DNA	87.525 ± 21.679	48.662 ± 31.202
Positivity, n (%)	7/7 (100)	8/8 (100)
ERc, fmol/mg protein	6.912 ± 3.699	5.913 ± 3.091
PgRc, fmol/mg protein	40.188 ± 25.683	21.897 ± 19.13

Enzyme Immunoassay

ERc and ERn were detected in all the prostatic samples of the untreated BPH patients examined. In this group, ERn concentrations were lower than those of the low-affinity SA class and higher than those of the high-affinity class. Also, in the treated group, ERn were detectable in all cases but the mean values were significantly lower than those of the untreated patients ($p < 0.01$; table 3).

PgR by EIA were higher in the nuclear than in the cytosolic fraction, and in the treated BPH patients mean concentration values were significantly lower than those found in the treated group ($p < 0.01$; table 3).

AR

ARn, evaluated in the BPH samples with the single-point SA, were positive in 6 of 10 untreated cases (153.183 ± 76.909 SD fmol/mg DNA) and negative in 9 of the 10 cases treated with *S. repens* extract, the only positive case showing levels of 108 fmol/mg DNA (table 2).

ARc were present in 9 of 10 prostatic tissue samples of untreated patients at a concentration of 23.878 ± 10.384 SD fmol/mg protein and in 6 of 10 treated cases at a concentration of 24.467 ± 12.697 fmol/mg protein, without significant differences between the two groups (table 2).

Conclusions

The results of the present investigation confirm the presence of ERc and ERn in human prostatic tissue, evaluated either by SA or by EIA.

According to SA, as described by other investigators [8–11], receptors were labelled by overnight incubation at 0–4 °C with tritiated estradiol in the presence or absence of an excess of DES, in order to correct the nonspecific binding: free steroids were removed by dextran-coated charcoal absorption and data were analyzed by Scatchard plots. Using this technique, multiple estrogen binding sites both in cytosol and nuclei were identified, in agreement with Ekman et al. [9]. One component corresponds to the high-affinity low-capacity binding, typical of an ER, which is measurable only at ligand concentrations below 1 n*M*. The 2nd component, the role of which is difficult to interpret, corresponds to the low-affinity high-capacity binding, measurable at ligand concentrations higher than 5 n*M*.

The single-point assay of estradiol binding, performed at ligand concentrations of 10 n*M* for ERn and 5 n*M* for ERc, refers to the 2nd component of the curvilinear Scatchard plot.

In our BPH tissue samples, ERc and ERn evaluated by SA or single-point assay were positive in almost all the cases of the untreated group: mean concentration values were higher than those reported by other investigators [9–11] who also found a low incidence of positivity, but were in agreement with those of Ghanadian and Auf [8] who used a similar technique. These controversial findings can be explained by the different ligand concentrations employed: our results and probably those of Ghanadian and Auf [8] coincide with the concentrations of the low-affinity high-capacity binding of SA.

ER-EIA was performed incubating the cytosolic and nuclear fraction with polystyrene beads coated with anti-ER monoclonal antibodies and then with an enzyme substrate solution to develop a color read spectrophotometrically. Using this method, ER were positive in the cytosolic and nuclear fraction of all the untreated samples, as found by Mobbs et al. [10], but the concentration values were intermediate, significantly higher than those of the 1st binding site component obtained by SA and lower than those of the 2nd component, probably because the antibody recognizes also receptor fragments, overestimating the levels of the 1st component.

The interesting finding of this study is that the group treated with *S. repens* extract was ERn negative not only when evaluated by a single-point assay, but also by SA, involving both the high- and low-affinity binding sites. On the contrary, ERc were comparable in treated and untreated groups.

These results were confirmed by those of EIA that showed ERn values of the treated group significantly lower than those of the untreated group, ERc being not modified.

Another data in favor of the inhibitory effect of the drug on the estrogen receptors can be obtained estimating PgR which were present in all the BPH samples exam-

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM

ined. The meaning of this receptor in the prostate is still obscure: however, in most estrogen-dependent tissues it has been demonstrated that the synthesis of PgR is a function of the estrogen receptors [13]. It derives that PgR measurement may be used as a marker of the estrogenic activity. Mobbs et al. [10, 14] have in fact observed a significant increase in PgR content in the R3327H lines of Dunning experimental rat prostatic tumor in response to estrogen treatment and in human prostatic carcinoma in response to DES diphosphate. On the contrary, both ER and PgR values were very low in orchiectomized patients.

The significant decrease in prostatic PgR concentrations of our treated group may be, therefore, the result of the ER inhibition by *S. repens* extract.

A possible explanation for this finding is that one or more fractions of *S. repens* extract are able to block by competition the translocation of ERc to the nuclei. A similar mechanism may be proposed for AR: in fact, whilst ARc were almost comparable in treated and untreated groups, ARn were found negative in 90% of our cases treated with *S. repens* extract.

This drug, therefore, appears to display an inhibitory effect both on AR and ER, probably because it is composed by several fractions, one of which with antiandrogenic action and another with antiestrogenic action. It cannot be excluded, however, that the primary effect is antiestrogenic and that the inactivation of AR and PgR and of the 5α-reductase activity is secondary to the ER blockade.

Whatever could be the mechanism of action of *S. repens* extract, its antiestrogenic effect is well documented, so that we may assume that this drug interferes at noncytotoxic doses with the molecular mechanisms involved in cell growth. Whether this action is uniquely mediated by the inhibition of the hormone interaction with the specific receptor protein has to be demonstrated.

The inhibition of the estrogen action may be pharmacologically applied to the treatment of BPH, since there is an increasing evidence that the primary role of androgens in the genesis of the disease may be conditioned by other factors, one of these being estrogens: in fact, it has been found that the androgen-dependent prostatic epithelial growth is stimulated by an estrogen-dependent proliferation of the stroma [14, 15].

So, one may assume that in the medical treatment of human BPH, inhibition of the estrogen action potentiates the effects of the antiandrogens. In fact, tamoxifen, a nonsteroidal antiestrogen, when administered to patients with BPH, is able to reduce stromal protein synthesis and stromal growth, the concentration both of ARn and ER, the 5α-reductase activity and the nuclear content of DHT [16–19].

Positive clinical results have been already reported by Di Silverio [20] with the combination therapy cyproterone acetate plus tamoxifen: in a randomized trial performed on 166 BPH patients, after 2 months of therapy, a 40–70% reduction in nicturia in 60% of the cases, a 30–70% reduction in daytime frequency in 65% of the cases and a 25–60% reduction in postmictional residue in 65% of the cases was found. The clinical response was better than that obtained with cyproterone acetate alone, but the prostatic volume, evaluated by rectal ultrasonography, was only slightly decreased (20% in 55% of the cases). Moreover, 78% of the patients complained of loss of libido and reduction of sexual performance, which represent the main problem of antihormone administration.

On the basis of these considerations, monotherapy with *S. repens* extract may be more favorably accepted, since on account of similar clinical results, when compared to the combination therapy cyproterone acetate plus tamoxifen; *S. repens* is well tolerated with a lower incidence of side effects.

References

1 Stenger A, Tarayre JP, Carilla E: Etude pharmacologique et biochimique de l'extrait hexanique de *Serenoa repens* B. Gaz Méd Fr 1982; 89:2041–2048.

2 Briley M, Carilla E, Fauran F: Permixon®, a new treatment for benign prostatic hyperplasia, acts directly at the cytosolic androgen receptor in the rat prostate. Br J Pharmacol 1983;79: 327–336.

3 Briley M, Carilla E, Roger A: Inhibitory effect of Permixon® on testosterone 5 reductase activity of the rat prostate. Br J Pharmacol 1984; 83:401–410.

4 Sultan C, Terraza A, Divillier C: Inhibition of androgen metabolism and binding by a liposterolic extract of *Serenoa repens* B in human foreskin fibroblasts. J Steroid Biochem 1984;20: 515–521.

5 Sultan C, Terraza A, Carilla E, Briley M, Descomps B: Anti-androgenic effects of Permixon: In vitro studies: in Di Silverio F, Steg A (eds): New Trends in BPH Etiopathogenesis. Roma, Acta Medica, 1988, pp 297–313.

6 Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem 1976;72:248–259.

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM

7 Burton K: A study of the condition and mechanism of the diphenylanine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1976;62:315–323.
8 Auf G, Ghanadian R: The characterization and measurement of cytoplasmic and nuclear oestradiol-17β receptor proteins in benign hypertrophied prostate. J Endocrinol 1982;93:305–316.
9 Ekman P, Barrack ER, Greene GL, Jensen EV, Walsh PC: Estrogen receptors in human prostate: Evidence for multiple binding sites. J Clin Endocrinol Metab 1983;57:166–176.
10 Mobbs BG, Johnson IE, Liu Y: Quantitation of cytosolic and nuclear estrogen and progesterone receptor in benign, untreated and treated malignant human prostatic tissue by radioligand binding and enzyme immunoassays. Prostate 1990;16:235–244.
11 Murphy JB, Emmott RC, Hicks LL, Walsh PC: Estrogen receptor in the human prostate, seminal vesicle, epididymis, testis and genital skin: A marker for estrogen responsive tissues? J Clin Endocrinol Metab 1980;50:938–949.
12 Vuhai MT, Logeat F, Warembourg M, Milgrom E: Hormonal control of progesterone receptors. Am NY Acad Sci 1977;286:199–209.
13 Mobbs BG, Johnson IE: Basal and estrogen-stimulated hormone receptor profiles in four R3327 rat prostatic carcinoma sublines in relation to hystopathology and androgen sensitivity. Cancer Res 1988;48:3077–3083.
14 Krieg M, Klotel G, Kaufmann J, Voigt KD: Stroma of human benign prostatic hyperplasia: Preferential tissue for androgen metabolism and estrogen binding. Acta Endocrinol 1981; 96:422–430.
15 Cunha GR, Chung LWK, Shannon JM, Tauchi O, Fujii H: Hormone-induced morphogenesis and growth: Role of mesenchymal-epithelial interactions. Recent Prog Horm Res 1983;39: 559–595.
16 Krieg M, Bartsch W, Thomsen M, Voigt KD: Androgens and estrogens: Their interaction with stroma and epithelium of human benign prostatic hyperplasia and normal prostate. J Steroid Biochem 1983;19:155–161.
17 Geller J, Liu J, Albert J, Fay W, Berry CC: Effect of antiandrogen and or antiestrogen blockade on human prostate epithelial and stromal cell protein. J Steroid Biochem 1986; 25:759–763.
18 Habib FK, Refati G, Robinson MRG, Stitch SR: Effects of tamoxifen on the binding and metabolism of testosterone by human prostatic tissue and plasma in vitro. J Endocrinol 1979; 83:369–378.
19 Albert JD, Geller J, Liu J, Faber LE: Tamoxifen decreases progesterone and nuclear androgen receptor in the human prostate. J Steroid Biochem 1984;21:659–662.
20 Di Silverio F, Tenaglia R: Anti-estrogens and anti-androgens in human benign prostatic hyperplasia; in Di Silverio F, Neumann F, Tannenbaum M (eds): Ipertrofia Prostatica Benigna. Amsterdam, Excerpta Medica, 1986, pp 117–125.

Congress Calendar

1.10.–3.10.1992
Monte Carlo
Monaco

Videourology 92: 4th World Congress

Information:
Convergences
Videourology 92, 120, avenue Gambetta
F–75020 Paris (France)
Fax: 33-1-40310165

1.10.–3.10.1992
Leysin
Switzerland

Annual Meeting of the Swiss Society of Urology

Information:
Prof. P. Graber (President)
Clinique de Chirurgie Urologique, Hôpital Cantonal
CH–1211 Genève 4 (Switzerland)
Prof. D. Hauri (Secretary)
Urologische Klinik, Universitätsspital
CH–8091 Zürich (Switzerland)

16.10.–17.10.1992
Wuppertal
FRG

Experimental Urology

Information:
Prof. Thüroff
Urologische Klinik
Klinikum Barmen, Heusnerstrasse 40
D-W–5600 Wuppertal 2 (FRG)
Tel.: 0202/8962263
Fax: 0202/8962139

Downloaded by:
University of Exeter
144.173.6.94 - 5/4/2020 8:57:31 AM