Antiproliferative Effect on Human Prostate Cancer Cells by a Stinging Nettle Root (*Urtica dioica*) Extract

Lutz Konrad[1,*], Hans-Helge Müller[2], Corinna Lenz[1], Helge Laubinger[1], Gerhard Aumüller[1], and Johannes Josef Lichius[3]

[1] Department of Anatomy and Cell Biology, Philipps-Universität Marburg, Marburg, Germany
[2] Institute for Medical Biometry and Epidemiology, Philipps-Universität Marburg, Marburg, Germany
[3] Institute of Pharmaceutical Biology, Philipps-Universität Marburg, Marburg, Germany

Received: March 18, 1999; Accepted: August 7, 1999

Abstract: **In the present study the activity of a 20% methanolic extract of stinging nettle roots (*Urtica dioica* L., Urticaceae) on the proliferative activity of human prostatic epithelial (LNCaP) and stromal (hPCPs) cells was evaluated using a colorimetric assay. A concentration-dependent and significant ($p < 0.05$) antiproliferative effect of the extract was observed only on LNCaP cells during 7 days, whereas stromal cell growth remained unaltered. The inhibition was time-dependent with the maximum of growth reduction (30%) at a concentration of 1.0E-6 mg/ml on day 5 compared to the untreated control. On day 4 and 6, the reduction in proliferation of LNCaP cells showed the minimal effective dose at 1.0E-9 mg/ml. No cytotoxic effect of ME-20 on cell proliferation was observed. The antiproliferative effect of ME-20 of stinging nettle roots observed both in an *in vivo* model and in an *in vitro* system clearly indicates a biologically relevant effect of compounds present in the extract.**

Key words: *Urtica dioica*, Urticaceae, benign prostate hyperplasia, epithelial prostate cells, stromal prostate cells.

Introduction

Benign prostate hyperplasia (BPH) is the most common disease of the prostate in elderly men and is characterised by the enhanced proliferation of the epithelial and/or stromal compartments (1). Pathological alterations caused by BPH in approximately 50% of all men above 70 years of age result in symptoms such as nycturesis and urinary retention. In Germany, plant-derived drugs are widely used in the treatment of initial stages of BPH (2), (3) and, although controversial, represent one of the most frequently used therapeutic approaches.

Rückle was the first physician who regularly used teas from stinging nettle roots in cases of urine retention (4). Since these first observations, various extracts of stinging nettle roots (*Urtica dioica* L., Urticaceae), were commonly used in the treatment of initial stages of BPH (5).

Planta Medica 66 (2000) 44 – 47

ISSN: 0032-0943

Based on our observations that various extracts from stinging nettle roots demonstrated proliferation-reducing effects in an *in vivo* mouse BPH system (6), (7), we decided to test a 20% methanolic extract (ME-20) in an *in vitro* proliferation assay, as this extract showed the highest effects *in vivo*. The *in vivo* mouse BPH system is characterised by a glandular hyperplasia based upon epithelial growth without any changes in the stromal compartment (7). In the present study, we examined the effect of ME-20 *in vitro* on the proliferative activity of human prostatic epithelial and stromal cells.

Materials and Methods

Plant material and extraction procedure

Dried roots of *Urtica dioica* were purchased from H. Finzelbergs Nachfolger (Andernach, Germany). Voucher specimens are deposited without number at the Department of Pharmaceutical Biology (Marburg, Germany).

20% methanolic-aqueous extracts were obtained from 1,005.8 g of dried, milled stinging nettle roots. Maceration was performed with 3.0 l of 20% methanol for 24 h at RT. The solution was then transferred to a percolation column and extracted with additional 5.0 l of 20% methanol. After lyophilization, 67.68 g methanolic extract similar to Bazoton® were obtained.

*Sugar analysis (Table **1**)*

Analysis of sugar content and composition was performed according to (8). Briefly, freeze-dried extracts after addition of myo-inositol as internal standard were hydrolysed with 2 M trifluoroacetic acid for 60 min at 121 °C. The hydrolysed material was reduced with $NaBH_4$ and alditols formed were acetylated prior to GLC analysis on a Permabond OV-225 fused silica column (25 m × 0.25 mm) using a FID. Uronic acid contents were determined by the biphenylol method (9).

Cell culture

2,000 LNCaP (**l**ymph **n**ode **ca**rcinoma of the **p**rostate; American Type Culture Collection) and 2,000 hPCPs cells (**h**uman **p**rimary **c**ulture of the **p**rostate **s**tromal compartment; generated in the Institute of Anatomy and Cell Biology, Marburg, Germany), respectively, were seeded in 96-well plates and

cultured in RPMI-1640 medium supplemented with 10% FCS (Linaris, Bettingen, Germany), 22.5 mM Hepes (Gibco BRL), 4.3 mM L-glutamine (Sigma, Deisenhofen, Germany), 100 µg/ml streptomycin sulfate, and 100 U/ml penicillin G (both from Biochrom, Berlin, Germany). The cells were cultivated without ME-20 (control) or once treated with various concentrations (1.0E-5 mg/ml to 1.0E-11 mg/ml) of ME-20 for 7 days. Each time point and concentration was determined in 8 wells. The whole experiment was repeated at least twice (n = 2).

Proliferation assays

Cell proliferation of LNCaP cells was measured with an MTT assay (10), and by determining the amount of genomic DNA by a fluorometer according to the manufacturer's recommendations (Pharmacia, Freiburg, Germany). Proliferation of hPCPs was determined by an amido black assay (11). The MTT assay and the amido black assay give similar results referring to the cell number (unpublished observations).

Cytotoxicity test

1.3 × 1.0E+4 cells were seeded in 24-well plates and incubated in RPMI-1640 supplemented with 10% FCS. After three days, the medium was replaced with one to which ME-20 at different concentrations (1.0E-5, 1.0E-7, 1.0E-9, and 1.0E-11 mg/ml) was added to the medium. After an 18 h incubation, the medium was discarded and the cells washed with PBS. Non-viable cells were stained with 0.4% trypan blue and, after an incubation of 5 min, counted with a microscope. As a negative control, RPMI-1640 medium without any drugs was used in the same way. As a positive control, cells were incubated 10 min at RT with 0.5 mM H_2O_2. Each experiment was performed in triplicate.

Statistical analysis

Primary endpoint for each concentration was the calculation of the means ± SEM of absorbance along the time points. The minimal effective dose (MED) was determined according to Williams (12). P values of < 0.05 were considered to be statistically significant.

Results

We tested ME-20 which is characterised by the following contents (6): proteins (49.5%), sugars (16.5%), β-sitosterol (0.49%), and lectin (0.03%). The sugar components were further characterised and demonstrated after total hydrolysis a high glucose content and moderate amounts of galactose and arabinose (Table **1**).

In comparison to the untreated control, LNCaP cells were administered with various concentrations of ME-20 and then treated as the control during 7 days. The resulting growth curves demonstrated that the ME-20-treated LNCaP cells are reduced in proliferation in comparison to the untreated control (Fig. **1**). In contrast, proliferative activity of stromal cells hPCPs remained unchanged (Fig. **2**).

In order to show the diminished proliferation of LNCaP cells more distinctly, we considered every value obtained for the control to be 100% for each day. The growth inhibition in the ME-20 treated groups was obtained by calculating the per cent reduction in proliferation in comparison to the untreated control (Fig. **3**). Proliferation of the androgen-dependent LNCaP cells was remarkably reduced in a concentration-de-

Table 1 Carbohydrate content and composition of ME-20.

	mg	Mol %
Rhamnose	0.0598	2.78
Arabinose	0.1929	7.68
Xylose	0.0510	2.04
Mannose	0.1016	4.85
Galactose	0.1928	9.19
Glucose	1.5386	73.45
Sample	10.23	
Uronic acids	0.1739	1.7

Neutral sugars determined by GLC [gas liquid chromatography (8)], uronic acids by spectrophotometric biphenylol-method (9).

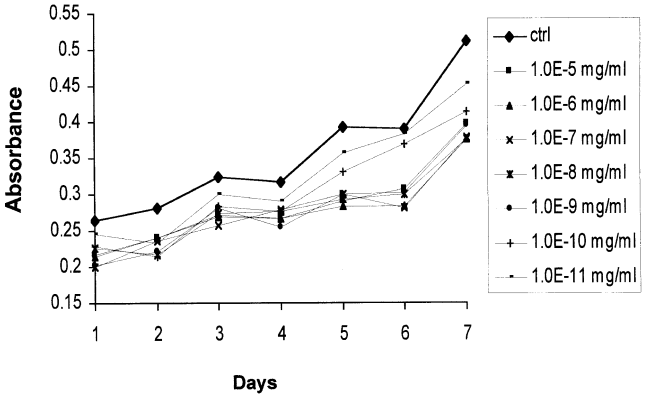

Fig. 1 Growth curves of LNCaP cells demonstrating the untreated control in comparison to the ME-20-treated cells. ME-20 was applied at different concentrations as indicated in Materials and Methods. Experiments were independently performed two times (n = 2) in 8 wells. (SEMs are not shown due to better clarity).

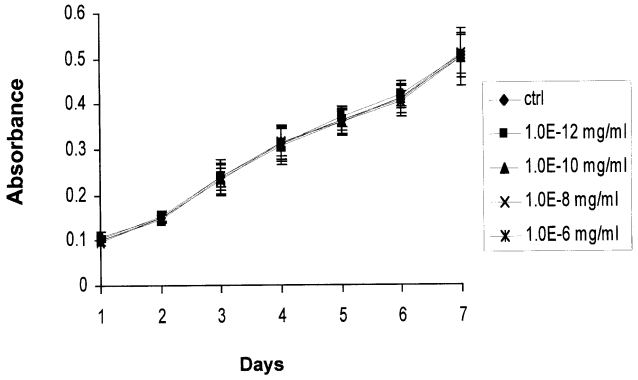

Fig. 2 Effects of the concentrations of 1.0E-6 to 1.0E-12 mg/ml of ME-20 on the growth of hPCPs cells. Proliferation of prostatic stromal cells was unaffected by ME-20. Each point represents the means ± SEM of three independently performed experiments (n = 3), obtained from 8 wells.

pendent manner, when ME-20 was added to the medium (1.0E-5 mg/ml to 1.0E-11 mg/ml). Suppression of epithelial cell proliferation could be observed starting on day 1 and reaching a maximum of approximately 30% of anti-proliferative activity on days 5 and 6 (Fig. **3**). The MED for days 4 and 6 was a concentration of 1.0E-9 mg/ml and 1.0E-11 mg/ml for days 5 and 7.

Three concentration-response curves for days 5, 6, and 7 of two independently performed experiments are shown as an example in Figure **4** underlining the specific effect of ME-20 on the proliferation of LNCaP cells.

The data of the MTT-assay were confirmed by the data obtained with the measurement of the genomic DNA (not shown). The DNA content was reduced by approximately 60% on days 5 and 6 at a concentration of 1.0E-6 mg/ml. No cytotoxic effect on cell proliferation was observed with the extract.

Discussion

The effect of stinging nettle root extracts on BPH is still controversially discussed, because the mechanisms of action remain unclear. In this study, the effects on prostate cells *in vitro* of a 20% methanolic extract of stinging nettle roots were investigated, an extract whose composition was defined by a standardised fractionating procedure. The results demonstrate for the first time a selective and significant proliferation-reducing effect on epithelial tumor cells, whereas the proliferation of the stromal cells remained unaffected. The inhibition of epithelial proliferation rate was time- and concentration-dependent.

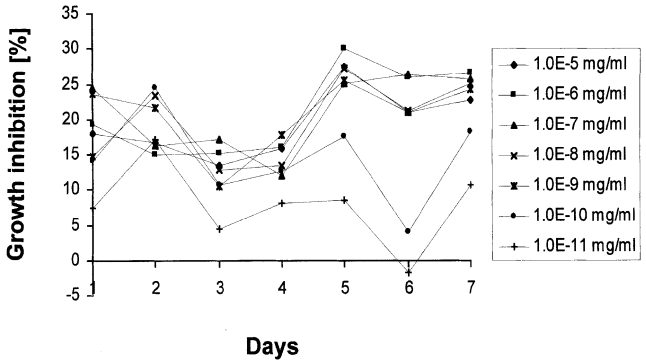

Fig. 3 Effects of the concentrations of 1.0E-5 to 1.0E-11 mg/ml of ME-20 on the growth of LNCaP cells. Growth inhibition was obtained by calculating the reduction in proliferation in comparison to the untreated control (= 100% proliferation). The time-dependent reduction is detectable reaching a maximum of approximately 30% on days 5 and 6. (SEMs are not shown because of better clarity).

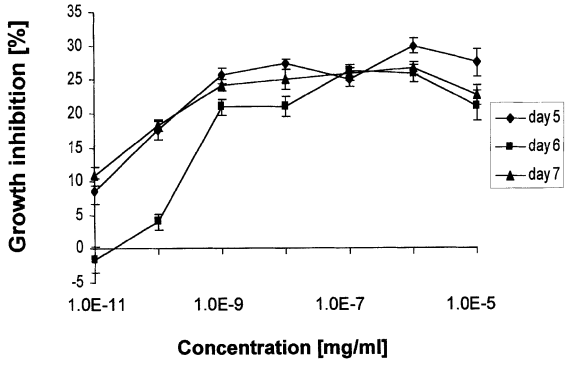

Fig. 4 Concentration response curves of LNCaP cells demonstrating the concentration-dependent reduction [%] in proliferation on days 5, 6 and 7 upon stimulation with varying concentrations of ME-20. The concentrations of 1.0E-5 to 1.0E-9 mg/ml are significantly ($p < 0.05$) different from the control. Each point represents the means ± SEM of two separate experiments (n = 2), obtained from 8 wells.

Our results are comparable with data obtained from *in vitro* studies using finasteride (Proscar®), a 5α-reductase inhibitor. Finasteride was shown to reduce proliferation of LNCaP cells after treatment (13). These inhibitory effects are mainly due to an inhibition of human 5α-reductase (14). In contrast, Bazoton® has neither anti-androgen nor 5α-reductase inhibitory activities (15).

Of the different modes of actions of stinging nettle root extracts already studied, the most accepted are presented below. Aromatase is a key enzyme in steroid hormone metabolism and mediates the conversion of androgens into oestrogens. A weak inhibition of the enzymatic activity was observed for some sterols and hydroxy fatty acids, however, these compounds are found at a very low concentration in *Urtica* drugs (16). Therefore, the effects of ME-20 on the mRNA expression of aromatase with semiquantitative RT-PCR in the stromal cells hPCPs were investigated, however, no effects were found (unpublished observation).

An aqueous extract of stinging nettle roots demonstrated a dose-dependent inhibition of the binding of sex-hormone binding globulin (SHBG) to its receptor (17). SHBG is a protein which reversibly and with high affinity binds androgens and oestrogens in plasma. Some lignans and their metabolites were shown to interfere with the binding of androgens to SHBG (18), thereby reducing the transport capacity for androgens.

In a clinical trial, β-sitosterol (2) significantly improved the symptoms and urinary flow of BPH patients. It must be kept in mind, however, that humans normally consume considerably larger amounts of sitosterol with their daily food.

UDA, another component of *Urtica* root extracts, was shown to directly inhibit cell proliferation of HeLa cells and to block binding of EGF to its receptor (19). In the same study a polysaccharide mixture from an aqueous root extract was shown to exert an anti-inflammatory activity in a rat paw oedema test. The results obtained in that study indicate that intact and partially hydrolysed polymeric sugar compounds can be resorbed and remain bioavailable (19). Preliminary experiments performed by us using a highly enriched polysaccharide fraction also significantly reduced the proliferation rate of epithelial LNCaP cells and revealed antiproliferative activity in the experimentally induced BPH mouse system (data not shown).

The results obtained with the *in vivo* BPH mouse model (6) and the observations in the present study using an *in vitro* approach with prostate cancer and stromal cell lines clearly showed a specific reduction in the proliferation of the epithelial cells by the 20% methanolic extract. Based on these obser-

vations, we conclude that the reduced prostate growth observed in the treatment of the *in vivo* induced BPH in mice with ME-20 is partly due to a specific inhibition of epithelial cell proliferation. Our results from both the present *in vitro* study and the previous *in vivo* study provide evidence that the antiproliferative effect of stinging nettle root extracts is biologically relevant and could be responsible for the beneficial outcome in early BPH treatment.

Acknowledgements

This study was partly sponsored by Kanoldt, Höchstädt, Germany.

References

[1] Shapiro E, Hartanto V, Perlman EJ, Tang R, Wang B, Lepor H. Prostate 1997; 33: 177 – 182
[2] Berges RR, Windeler J, Trampisch HJ, Senge T, and the β-sitosterol Study Group. Lancet 1995; 545: 1529 – 1532
[3] Buck AC, Cox R, Rees RW, Ebeling L, John A. Br. J. Urol. 1990; 66: 398 – 404
[4] Rückle E. Hippokrates (Informationen aus der Medizin für naturgemäße Lebens- und Heilweise, Wissenschaft und Praxis) 1950; 21: 55 – 56
[5] Engelmann U, Boos G, Kres H. Urologe [B] 1996; 36: 287 – 291
[6] Lichius JJ, Muth C. Planta Med. 1997; 63: 307 – 310
[7] Chung LW, Matsuura J, Rocco AK, Thompson TC, Miller GJ, Runner MN. Ann. N. Y. Acad. Sci. 1984; 438: 394 – 404
[8] Blakeney AB, Harris PJ, Stone BA. Carbohydr. Res. 1983; 113: 291 – 296
[9] Blumenkrantz N, Asboe-Hansen G. Anal. Biochem. 1973; 54: 484 – 498
[10] Mosmann T. J. Immunol. Methods 1983; 65: 55 – 63
[11] Schulz J, Dettlaff S, Fritzsche U, Harms U, Schiebel H, Derer W, Fusenig NE, Hülsen A, Böhm M. J. Immunol. Methods 1994; 167: 1 – 13
[12] Williams DA. Biometrics 1972; 28: 519 – 531
[13] Bologna M, Muzi P, Biordi L, Festuccia C, Vicentini C. Urology 1995; 45: 282 – 290
[14] The Finasteride Study Group. Prostate 1993; 22: 291 – 299
[15] Rhodes L, Primka RL, Berman C, Vergult G, Gabriel M, Pierre-Malice M, Gibelin B. Prostate 1993; 22: 43 – 51
[16] Ganßer D, Spiteller G. Planta Med. 1995; 61: 138 – 140
[17] Hryb DJ, Khan MS, Romas NA, Rosner W. Planta Med. 1995; 61: 31 – 32
[18] Ganßer D, Spiteller G. Z. Naturforsch. 1995; 50c: 98 – 104
[19] Wagner H, Willer F, Samtleben R, Boos G. Phytomedicine 1994; 1: 213 – 224

Dr. Lutz Konrad

Department of Anatomy and Cell Biology
Philipps-University
Robert-Koch-Str. 6
D-35033 Marburg
Germany
E-mail: Konrad@mailer.uni-marburg.de
Fax: +49-(6421)-28-65783
Phone:+49-(6421)-28-64004