

Experimental Treatment Studies with Cernilton® in Human Benign Prostatic Hyperplasia

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Introduction

Despite the high incidence of benign prostatic hyperplasia (BPH), so far a conservative treatment modality has not been established internationally (4,5). The reasons for this are the multifactorial etiology of the symptoms in patients with BPH (5) and the lack of suitable animal models to elucidate the pathogenesis of BPH (5,9). This makes the search for conservative therapies aimed at the underlying causes of the disease process difficult. Furthermore, all clinical trials in patients with BPH are complicated by a very strong placebo effect. Currently, patients with BPH up to stage III according to Vahlensieck are treated conservatively with phytotherapy in Germany (11,12).

To address some of the problems outlined above we established the heterotransplantation of human BPH tissue in nude mice as a model (Fig. 1) to evaluate the etiology of BPH and to facilitate the investigation of drug therapies and their mechanisms (8,13). In the context of these studies we utilized the phytopharmakon Cernilton® (Extract. pollinis sicc.) since it had shown significant effects in placebo-controlled clinical trials (2,3). Our experimental studies were planned to address the question whether in the nude mice model a significant growth-inhibiting effect in hormonally stimulated human BPH was measurable.

Materials and Methods

The NMRI nu/nu mice were kept under sterile conditions at 27°C and a relative humidity of 55%. They were fed a standard diet of Altromin (Lage, Germany) and water ad lib. Human BPH tissue was obtained by open transvesical prostatectomy from a patient with histologically proven BPH and divided in small pieces under sterile conditions after representative sections had been submitted for histology. Within one hour, 3 x 3 x 3-mm large pieces of tissue were transplanted subcutaneously on both sides of the thorax in 3-months-old male NMRI nu / nu mice which had been castrated the day before.

Hormonal stimulation was affected by silicon implants containing 5 (x-dihydrotestosterone DHT) and estradiol (E2) as described by van Steenbrugge (10). The animals were divided in three groups with 4 animals each (= 8 tumours). Groups II and III received the implants with DHT (serum levels of DHT 8 ng/ml) and E2 (serum level of E2 400 pg/ml) for hormonal stimulation, while group I served as controls (serum levels of DHT and E2 not measurable). The mice in group III were additionally treated with the pollen extract Cernilton® (Extract. pollinis sicc., 2,5:1 which was given orally through a feeding tube as 10 mg / 25 g body weight twice weekly.

The tumor size was assessed by measuring the diameters and calculating the volume according to the following formula: length x width ²/2 (7).

After 6 weeks the animals were sacrificed and the tissue removed for histology. A semiquantitative determination of the human LDH isoenzymes (electrophoresis) was planned



6 weeks after transplantation to determine the human origin of the tissue.

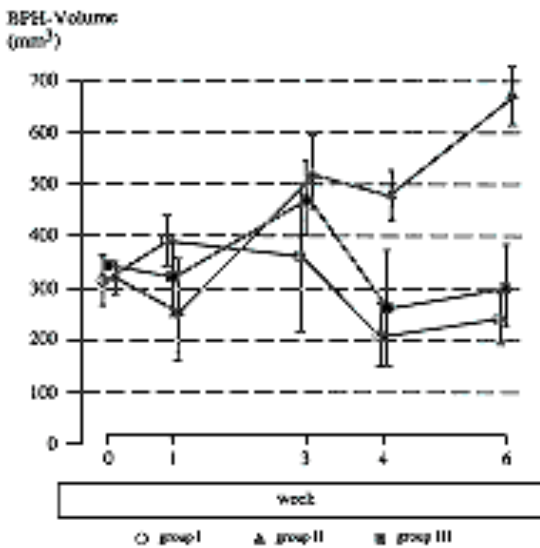


Fig. 2 Growth curves ($\bar{x} \pm s$) of the human BPH transplants (nude mouse model) in the group without stimulation (1), with hormonal stimulation (11), and with hormonal stimulation and Cernilton® treatment (I 11) (for details, see "Materials and Methods").

Statistical calculations were done to proof the experimental model, to test for homogeneity and for treatment effects. The t-test was used to compare mean values in two treatment groups, a one-way analysis of variance to compare mean values between all three groups, and an analysis of variance for the repeated measurement design. When all volume measurements were considered, the correlation between the two tissue pieces in each mouse showed a very strong correlation. Therefore the side related measurements were not considered an independent variable but interpreted as double measurements.

Results

The BPH tissue 6 weeks after transplantation is in all cases histologically vital and shows no sign of necrosis or rejection.

Immediately after transplantation the BPH tissue volumes are comparable in all groups ($p = .605$).

The growth curves of the BPH volumes are markedly different over the 6 weeks duration of follow-up (Fig. 2): in group I (control) the volume decreases according to the expectation, while in group II with hormonal stimulation by DHT and E2 an average increase in volume of 354.7mm³ is noted. A comparison between these two groups yields significant differences in particular at week 4 and 6 (Table 1). The validity of the animal model is therefore established.

In group III (hormonal stimulation with DHT and E2 and treatment with pollen extract) a slight decrease in volume in comparison to the starting volume is noted after 6 weeks, which is significantly different from the mean volume at week 6 in group II (control treated animals) ($p = .003$) (Table 2). At no time there are any significant differences between group III (Cernilton®-treated and hormonally induced) and the control group I (Table 3). Analysis of variance reveals a significant difference of the mean at all four measurement points between the two hormonally treated groups ($p = .007$) demonstrating a growth inhibition of the pollen extract treated animals (group III).

Tab. 1 Volume differences ($\bar{X} \pm s$) of the human BPH transplants (nude mouse model) in comparison with the starting volume in the groups without stimulation (1) and with hormonal stimulation (11). Statistical analysis demonstrated the effectiveness of the animal model (for details, see "Materials and Methods").

Time point of control	Group I \bar{x}	s	Group II \bar{x}	s	Validation of animal model p-value
1. week	74.5	95.3	-62.3	67.1	0.076
3. week	47.3	103.0	203.8	50.4	0.046
4. week	-102.1	106.1	151.5	57.2	0.008
6. week	-69.6	71.6	345.7	69.5	0.001

Tab. 2 Volumes ($\bar{X} \pm s$) of the human BPH transplants (nude mouse model) in the groups with hormonal stimulation (11) and with hormonal stimulation and Cernilton® treatment (111). Statistical analysis revealed a significant difference after 6 weeks in a time-related comparison between the two groups (for details, see "Materials and Methods").

Time point of control	Group II \bar{x}	s	Group III \bar{x}	s	Group III vs. Group II p-value
before treatment	318.3	32.7	343.0	6.1	
1. week	256.0	99.5	324.8	78.1	0.516
3. week	522.2	75.0	473.5	72.8	0.208
4. week	479.8	48.0	262.8	112.5	0.047
6. week	673.0	58.4	307.0	79.3	0.003

All examined specimens show histologically an epidermoid metaplasia (Fig. 3). There is no

histological difference between the two

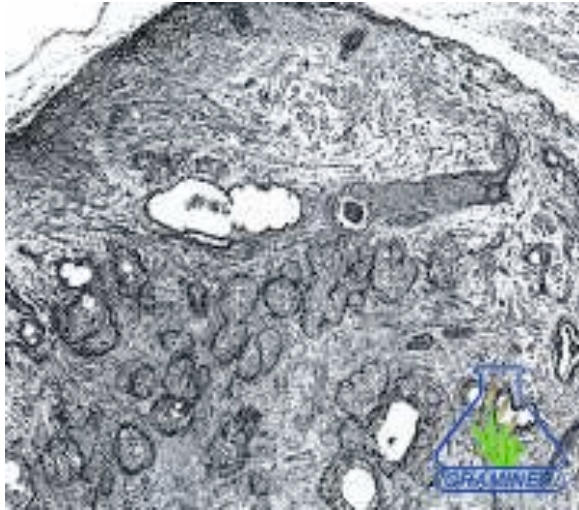


Fig. 3 BPH tissue 6 weeks after transplantation into the nude mouse and hormonal stimulation with DHT and E2 (for details, see "Materials and Methods") (HEX250)

treatment groups.

Discussion

The results of this study demonstrate a significant growth-inhibiting effect of orally administered Cernilton® on heterotransplanted human BPH tissue in nude mice after 6 weeks of treatment under conditions of hormonal stimulation by DHT and E2. The model was validated and it can therefore be concluded that for the first time a growth-inhibiting effect of a phytopharmakon on human BPH tissue is demonstrated experimentally.

To what extent these results have clinical relevance as a therapeutic principle in patients with BPH cannot be answered definitely. Both stimulating hormones DHT and E2 are given in relatively high doses, and the amount of pollen extract given exceeds that usually given to patients by a factor of 50. While this is done to allow the effect to take place in the relative short time span of 6 weeks, extrapolation of the data obtained to other experimental or therapeutic in vivo situations is not possible.

The pollen extract group starts to show a significant difference from the also hormonally treated control group 11 after about 4 weeks. The clinically observed effects on voiding symptoms, residual urine and prostate volume (2,3) indicate positive changes within the first 6 weeks, and therefore there is no discrepancy between the human and the experimental data.

The mechanism of action cannot be determined from our observations since no histological differences were found between the treated groups. Since DHT and E2 were supplied, the growth inhibition cannot be the result of an inhibition of the 5 α -reductase or aromatase, which are target enzymes of innovative drug treatments for BPH (1). It is possible that the prostaglandin and leukotrienbiosynthesis in the prostate is influenced by the pollen extract (6).

Investigations in rats (10) and dogs (14) have contributed greatly to our understanding of the hormonal mechanisms involved in the etiology of BPH. However, it must be remembered that the rat does not develop spontaneous BPH and that dog BPH differs greatly in its histological characteristics from human BPH. Since in the nude mice model human BPH tissue retrieved at open prostatectomy is utilized, the observed effect caused by Cernilton® may resemble the situation in humans more closely.

In summary, the nude mice model described here appears to be useful in experimental studies of the etiology of BPH as well as the mechanisms of effect of drug treatments for BPH. Further investigations utilizing the pollen extract in this model could serve to elucidate better its pharmacodynamic mechanism of action.

Tab. 3 Volumes ($X \pm s$) of the human BPH transplants (nude mouse model) in the groups without stimulation (1) and with hormonal stimulation and Cernilton® treatment (111). Statistical analysis revealed no significant differences after 6 weeks in a time-related comparison between the two groups (for details, see "Materials and Methods").

Time point of control	Group I \bar{x}	s	Group III \bar{x}	s	Group III vs. Group I p-value
before treatment	315.9	48.7	343.0	6.1	
1. week	390.4	50.8	324.8	78.1	0.231
3. week	363.1	141.9	473.5	72.8	0.255
4. week	213.8	63.7	262.8	112.5	0.809
6. week	246.3	54.0	307.0	79.3	0.595

Summary

The mechanism by which human BPH is induced is unresolved. As a result there is currently no established conservative treatment option available for patients with BPH. Up to stage III according to Valdensieck phytotherapy is commonly used as conservative treatment.

We established the heterotransplantation of human BPH tissue in athymic nude mice (NMRI nu / nu mice) as a model to investigate the etiology of BPH as well as the possible mechanisms of therapeutic approaches. The study presented here was designed to test whether the phytopharmakon Cernilton® has a measurable effect on the volume of the transplanted BPH tissue in this model.

Human BPH tissue was grafted on NMRI nu/nu mice. The mice were stimulated by means of silicon implants containing dihydrotestosterone (DHT) and estradiol (E2). In comparison with the non-stimulated controls, a significant increase in volume was noted ($p = .001$). Cernilton® was tested in this model and induced a significant growth inhibition of the BPH tissue in comparison to the hormonally stimulated control group ($p = .007$). There were no histological differences noted. In all cases the tissue was vital 6 weeks after transplantation.

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