



Inhibition of Growth of Human Benign Prostatic Hyperplasia by Cernilton N in the Nude Mouse Model

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Introduction

In spite of the high incidence of benign prostatic hyperplasia (BPH) a conservative therapy for this disease has still not been established (1,2). The reasons for this are the following: On the one hand, the symptomatology in patients with BHP is due to various different factors, which leads to uncertainty regarding the objective parameters to be considered in clinical trials (2); also, all clinical investigations of the treatment of BPH present the problem of a high placebo effect. On the other hand, the aetiology of prostatic hyperplasia is still unexplainable, due to lack of suitable experimental models (2,3). This makes the search for casual factors on which to base a conservative therapy difficult.

At present, mainly phytotherapeutic agents are used for conservative treatment up to Stage II of the disease according to the classification of Vahlensieck (4, 5). We have established the heterotransplantation of human BPH tissue into the nude mouse as a model for the evaluation of the aetiology of BPH and of drug therapies and their mechanisms (6). Within the framework of these investigations we have studied the plant-based preparation Cernilton N in this model, since in a clinical trial it had been possible to show a significant effect with Cernilton N in comparison with placebo (7). The aim of these first investigations is to answer the question whether a significant effect on the growth of a hormonally stimulated BPH can be demonstrated in our model.

Materials and Methods

All the NMRI nu/ nu mice are kept in a special laboratory, under sterile conditions with a constant relative humidity of 55% and a constant temperature of 27 C. They receive a standard diet of Altromin (Lage) and water.

The human BPH tissue is obtained, by the transvesical prostatectomy, from two patients with BPH.

The tissue is cut into small pieces under sterile conditions and reference tissue is kept on one side for the histological assessment.

Within one hour, pieces measuring 3x3x3 mm are transplanted into both sides of the thorax of the mice.

The test animals are three-month-old male NMRI nu/ nu mice, orchietomized one day previously.

At the same time, silicone implants with 5-alpha-dihydrotestosterone (DHT) and oestradiol (E₂) are implanted subcutaneously, for hormonal stimulation of the tissue, as described by Steenbrugge (8).

Three groups, each of 4 animals (= 8 tumours), are formed per tumour-line. Groups II and III receive the silicone implants with DHT (serum level for DHT: 8.0 ng/ ml) and E₂ (serum level for E₂: 400 pg/ ml) for the hormonal stimulation. Group I serves as control (serum levels of DHT and E₂ below the measurable levels):

The mice of Group I are also treated with the pollen extract, Cernilton N (Extract. pollinis sicc. 2.5:1), in the dose of 10 mg/25 g body weight, twice a week, p.o., through stomach tube. Based on body weight, this dosage is equivalent to 50 times the dosage in humans (in order to obtain a speeded up effect). The size of the tumours is measured once a week, with a calliper. Their volume is calculated by means of the formula,

length of tumour x width of tumour ²/₂, as described earlier (9).

After 2 months the animals are sacrificed and the tissue removed for histological examination.

The human character of the tissue is checked by semi-quantitative determination of the human LDH isoenzymes (electrophoresis), also 2 months after the heterotransplantation.

Statistical analyses are performed by and independent investigator, whereby the t-test is used for comparison of mean values in 2 comparative groups and a one-way analysis of variance for comparison of the mean values in 3 comparative groups.

Results

In all cases the BPH tissue was histologically vital two months after transplantation, with no signs of necrosis or rejection.

In group I (control group) the volume of the tumours did not change significantly during the two months in the body of the mouse.

In the other two groups the volume of the transplanted tumours increased in the course of the two months. In comparison with the control group this increase in volume is statistically significant ($p < 0.05$). The increase observed in Group III (treatment-group) is, however, significantly less than that in Group II ($p < 0.008$).

The volumes of the transplanted prostate tissue before and two months after transplantation are shown in Table 1 and the growth curves are presented in Figure 1.

All the transplanted prostate-tissue preparations show an epidermoid metaplasia. A difference between the two groups with hormonal stimulation could not be demonstrated histologically.

Discussion

A statistically significant inhibition of growth through the application of Cernilton N can be demonstrated for human BPH in the nude mouse model. This result concurs with the results of the clinical trial (7). On the other hand, however, it must be born in mind that the

transferability of these findings to man is limited. The doses of the stimulant hormones on the one hand and of the therapeutic agent on the other, which are used in order to achieve the speeding-up effect, are both unphysiologically high. Also, in man the size of the prostate alone is insufficient to explain the whole pathological picture (2).

A conclusion regarding a possible mechanism of action cannot be drawn on the basis of our results. Also the histological picture, due to the lack of differences between groups, provides no information on the mechanism of the inhibition of growth of the tumour. However, an inhibition of the enzymes, 5-alpha-reductase and aromatase, can be excluded, since the end-products of the biochemical reactions catalyzed by these enzymes are substituted.

The model described here is nevertheless suitable, through further investigations of the prostate tissue, to contribute to clarification of the mechanisms of action.

Cernilton N is capable, under experimental conditions, of exerting an objectively evaluable effect on the growth of human BHP tissue.

References

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Table 1: Volume of BPH – Mean values (MV) Sunday 29.4.90, 20.36 hrs

| Time | Group I MV | Group II MV | Group III MV | Group I s | Group II s | Group III s | p |
|----------|------------|-------------|--------------|-----------|------------|-------------|-------|
| Baseline | 343.0 | 318.3 | 315.9 | 6.1 | 32.7 | 48.7 | 0.940 |
| 1st week | 324.8 | 256.0 | 390.4 | 78.1 | 99.5 | 50.8 | 0.076 |
| 3rd week | 473.5 | 522.2 | 363.1 | 72.8 | 75.0 | 141.9 | 0.046 |
| 4th week | 282.8 | 479.8 | 213.8 | 112.5 | 48.0 | 63.7 | 0.008 |
| 6th week | 307.0 | 673.0 | 246.3 | 79.3 | 58.4 | 54.0 | 0.001 |

Figure 1 : Volume of the benign prostatic hyperplasia after heterotransplantation

