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Effect of Heat Exposure on Viability and Contractility of Cultured Prostatic Stromal Cells

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Key Words

Thermotherapy · Benign prostatic hyperplasia · Stromal cell

Abstract

Objectives: Different thermotherapeutic modalities such as transurethral microwave therapy or transurethral needle ablation have been developed to provide effective alternatives to surgical management of benign prostate hyperplasia (BPH). The mechanisms of thermotherapy, however, are not completely understood. We developed a model to investigate the effects of heat application on stromal cell viability and contractility.

Methods: Cells isolated from prostatectomy and cystoprostatectomy specimens were cultured in a selective medium. Temperatures ranging from 37 to 50°C were applied for 1 h. Cell contraction was visualized by means of a cell culture microscope equipped with a time-lapse video system. For quantitative analysis, the percentage of contracting cells was evaluated; 10 μ*M* of phenylephrine were applied for adrenergic stimulation of the cells.

Results: On immunohistochemistry and phase-contrast microscopy, these cells were identified as prostatic myofibroblasts. Incubation at 50°C for 1 h in vitro induced immediate death of all cells, whereas at 45°C all cells survived. At 37°C 55% of the cells were seen to contract after addition of phenylephrine. Immediately after incubation at 45°C contraction rate decreased to 29%, but returned to 46% 1 day later.

Conclusions: With this model, it is possible to study the mechanisms of thermotherapy in vitro. The results suggest that the effects of thermotherapy are due to the induction of cell death rather than to reduced stromal cell contractility. Furthermore, the data show that treatment is probably only successful if temperatures in excess of 50°C are maintained.

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Introduction

BPH is one of the commonest diseases in elderly men. In BPH, urethral obstruction results from a static component (i.e. enlargement of the prostate) as well as a dynamic mechanism (i.e. increased contractility of prostatic smooth muscle cells). Various therapeutic modalities have been developed to counteract these two phenomena. Volume reduction can be achieved by surgical procedures such as transurethral resection of the prostate, whereas smooth muscle cell contractility is reduced by α_1-adrenoceptor antagonists.

More recently, various modalities of thermal therapy, e.g. transurethral microwave therapy (TUMT), transurethral needle ablation (TUNA) and others, have been introduced for the treatment of bladder outlet obstruction secondary to benign prostate hyperplasia (BPH) [1–3]. The mechanisms of thermotherapy, however, are still not entirely understood. Clinical studies have demonstrated that microwave therapy, for example, does not significantly reduce the size of the prostate in BPH patients [4]. Hence, it was assumed that thermotherapy acts by reducing prostatic smooth muscle cell contractility [5]. The aim of the present study was to develop a model for investigating prostatic stromal cell contractility and viability after heat application.

Material and Methods

Cells were isolated from human prostatic tissue obtained from 10 prostatectomy and cystoprostatectomy specimens of BPH, prostate cancer and bladder cancer patients. The prostates were dissected by a standardized technique, and tissue was taken from the periurethral zone. Specimens from cancer patients were examined histologically by frozen section to exclude tumor invasion of the tissue from which the cells were taken. Only small samples were taken so as not to interfere with tumor staging and grading by the pathologist. Because of the small amount of tissue available, the explant culture technique was used. The tissue was cut into small pieces which were subsequently attached to the bottom of a cell culture flask (Falcon). Then, the culture medium was added, which consisted of modified MCDB-131 with 15% dialyzed horse serum, 10 m*M* of HEPES, pH 7.2, 2% penicillin-streptomycin solution and 2% MEM-EAGLE nonessential amino acids. Furthermore, 5 μg/ml of insulin, 10 μg/ml of transferrin (Boehringer, Mannheim, Germany), 5 ng/ml of sodium-selenite, 0.1 μ*M* of estradiol and 0.1 μ*M* of dexamethasone (Sigma Chemical Company, St. Louis, Mo., USA) were added. This culture medium was previously shown to stimulate the growth of smooth muscle cells and to suppress the growth of fibroblasts [6]. The cells were incubated at 37 °C in 5% CO_2 and 95% humidity. The first cell passage with trypsin/EDTA was performed once approximately 50% of the bottom of the culture flask were covered with cells. Further passages were done at intervals of 5–10 days depending on the growth rate. Immunohistochemical staining was performed to characterize the cells. Antibodies to smooth muscle cell a-actin, myosin, desmin and vimentin (Sigma) as well as an antifibroblast antibody (Dianova, Hamburg, Germany) were used. The procedure has been described in previous publications [7]. A prostatic carcinoma cell line (LNCaP) and genital skin fibroblasts served as controls.

For video microscopy, the bottom of the culture flasks was coated with the viscous agent Cell-tak (Collaborative Biomedical Products, Bedford, Mass., USA) in a concentration of 4 μg/cm^2. Observation was performed with a cell culture microscope at ×200 magnification. A videosequence was performed from a previously selected field within the cell layer in each particular experiment by means of a videorecorder connected to the microscope. Because of the low velocity of cell contractions, a time-lapse video system was mandatory. With this system, an observation of cell contractions in sequences representing approximately 1 s real time per frame was possible. A number of 12–25 (mean 16) cells were observed within each microscopic field under investigation. Constant physiological conditions were maintained throughout the experiment by an incubation chamber around the microscope. The temperature could be set between 20 and 55 °C. In heat exposure experiments, the chamber was heated to temperatures of 45 or 50 °C. The cell culture flask was placed into the chamber before starting video observation. Heat exposure at a constant temperature was continued for 60 min. Following heat exposure, all cells in the culture flask were investigated morphologically. Video monitoring was continued as long as there were viable cells. Experiments in which all cells became necrotic were stopped. The temperature was downregulated to 37 °C after 1 h of heat exposure. Then the adrenergic contraction stimulant phenylephrine was added to the culture flask. The agent was dissolved in culture medium to obtain an end concentration of 10 μ*M*. In this concentration, phenylephrine has previously been shown to effectively induce stromal cell contractions [7]. Phenylephrine was added to the culture medium immediately after heat exposure, and the cells were observed for another 2 h. Control experiments with cultures from the same cell strain were performed prior to heat exposure studies. In control experiments, the cells were incubated at 37 °C throughout the experiment. After an initial 60-min observation period, 10 μ*M* of phenylephrine were added and video microscopy continued for another 2 h. In sham experiments, cells were observed for 3 h at 37 °C using a culture medium containing no stimulants.

For quantitative analysis, the total number cells in the observation field as well as the number of contracting cells were evaluated. Results are presented as percentage of contracting cells. For statistical analysis, the Kruskal-Wallis test (Abacus Concepts, Berkeley, Calif., USA) was used. Data are presented as mean values with standard deviations.

Results

With this culture model, a first cell passage was performed after 2 weeks. After 2–3 passages, only cells with stromal morphology were found to be present in the culture. The experiments were initiated after the 3rd or 4th passage, when the cells exhibited sufficient contractility. Seven to 9 passages could be performed successfully in most cell strains. Immunohistochemical staining was positive for

smooth muscle cell α-actin, myosin and desmin. However, a weak positive staining was also observed for the antifibroblast antibody, thus identifying these cells as myofibroblasts, an intermediate form between smooth muscle cells and fibroblasts. Genital skin fibroblasts were negative for the muscle cell markers, but positive for the antifibroblast antibody, whereas LNCaP cells were negative for all smooth muscle cell markers. Immunohistochemical documentation has been presented in previous publications [6, 7].

Morphologically, the cells remained unchanged after incubation at 37 and 45 °C for 1 h. No cell death was detectable by means of video microscopy. The cells also remained viable the following days after heat exposure, and further cell passages could be performed successfully. If incubated at 50 °C, however, all cells began to shrink after 30 min, and by the end of the experiment only condensed spots of the cells were left (fig. 1). No further cell passages could be performed since the cells did not recover any more.

Under the experimental conditions described, cell contractions could be well documented by video microscopy (fig. 2). Contractions were seen as shortenings of the cells and could be clearly distinguished from other cell movements like pseudopadal extension, which were also recognized during video observation. Contractions were seen until the 5th day after cell passage; they were slow and could therefore be observed with the help of the time-lapse system only. There was a great variability in contractility between different cell strains. Only cell strains with a contraction rate of at least 25% after adrenergic stimulation in control experiments were used for the present study. At 37 °C, 18% of unstimulated cells were seen to contract during the 2-hour observation period (fig. 3). After addition of 10 μ*M* of phenylephrine, the contraction rate increased significantly (55%). The first contractions were noted 5–30 min after the application of the agent. The observation period was not prolonged more than 2 h because at that point of time, the contraction rate began to decrease slowly. At 45 °C, cell contractility was found to decrease significantly (29%). However, this was only a transient effect which was observed in the initial phase after heat exposure. Twenty-four hours after heat application, the contraction rate returned to 46% and remained unchanged after further cell passages. In none of the experiments was acontractility observed in viable cells at temperatures lower than 50 °C. Cells irreversibly damaged following incubation at 50 °C for 1 h did not exhibit any contractility upon stimulation with phenylephrine. Genital skin fibroblasts and LNCaP cells showed pseudopadal extension, but no contractions at all.

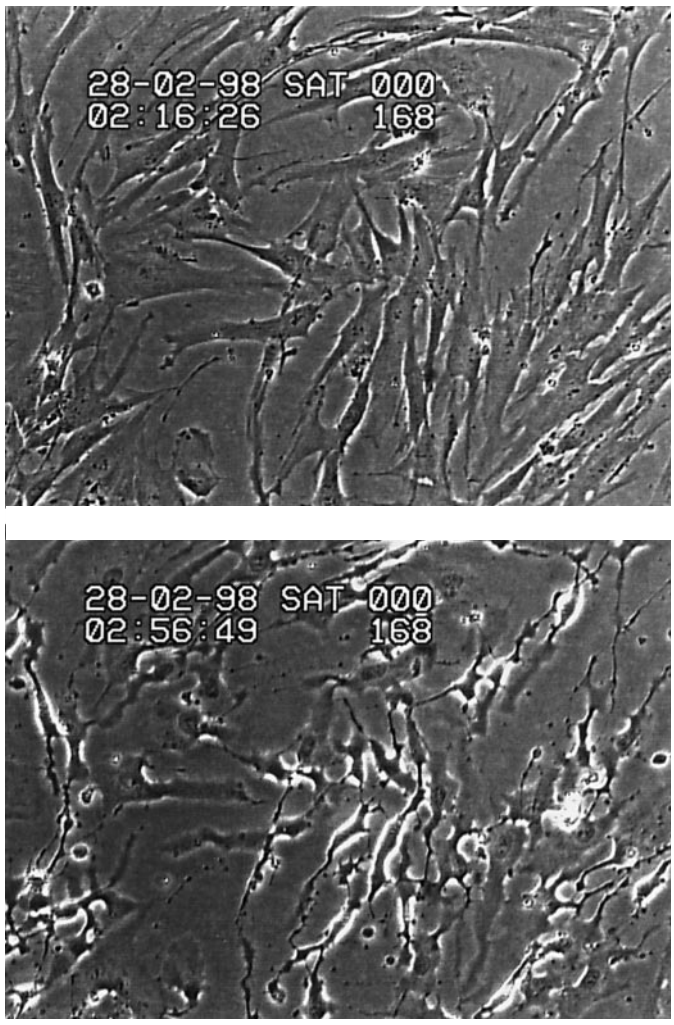

Fig. 1. Effect of thermotherapy on viability of prostatic stromal cells as demonstrated by means of videomicroscopy. At 50 °C all cells became necrotic within 60 min. **a** Start of videomicroscopy. **b** Sixty minutes after heat exposure.

Discussion

The stromal compartment, i.e. smooth muscle cells, fibroblasts and connective tissue, constitutes a major component of the prostate. Morphometric analysis demonstrates that BPH is primarily a disease of the stromal tissue [8]. In BPH, bladder outlet obstruction results from two different mechanisms, first a static component, i.e. an enlargement of the prostate, and second a dynamic one, resulting from the increased contractility of prostatic smooth muscle cells. Prostatic smooth muscle tone is known to be mediated primarily by α_1-adrenergic stimulation. Various models of prostatic stromal cell cultures have been described. The culture model of the present study was described previously [6, 7].

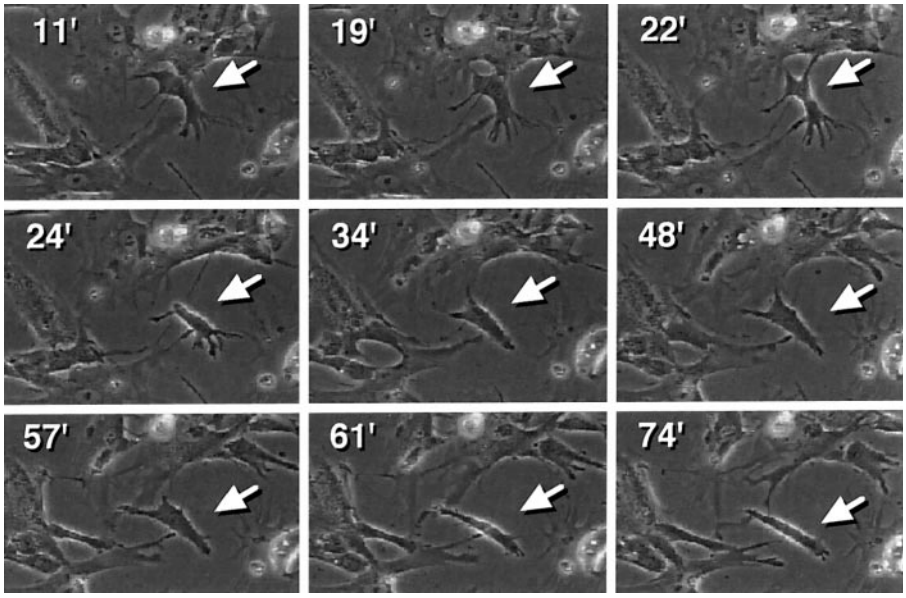

Fig. 2. By means of videomicroscopy cell contractions could be documented. The video sequence demonstrates contraction of the cell marked by arrows.

Immunohistochemical analysis suggests that the cultured cells are myofibroblasts, an intermediate form between smooth muscle cells and fibroblasts. These cells are known to differentiate into either smooth muscle cells or fibroblasts and to possess contractile properties [9]. Complete differentiation into smooth muscle cells was probably not possible within a period of 3 days after cell passage. Previous studies have shown that it takes these cells at least 4–6 days to express highly specific muscle cell markers such as the smooth muscle myosin heavy chain. However, it was not possible to prolong the interval between cell passage and experiment, otherwise the cells would have become too firmly attached to the culture flask and thus would have been unable to contract. A major aim of the present study was to develop a model of prostatic stromal cell contractions for video microscopy. In a previous study, this model was successfully used to investigate the effect of α_1-adrenoceptor agonists and antagonists [7]. Phenylephrine was shown to be a potent stimulant of prostatic stromal cell contraction. In the present study, 10 μM of phenylephrine was applied, because this concentration proved to be most effective, leading to a contraction rate of 55%.

The effect of thermotherapeutic modalities such as TUMT or TUNA is still not completely understood. Clinical studies have shown that microwave therapy for example does not act primarily by reducing the volume of the prostate [4, 10]. Histologic investigations have demonstrated that TUMT induces necrosis of prostatic cells [4, 11–13]. These authors describe uniform hemorrhagic necrosis, tissue devitalization and periurethral thrombosis without significant inflammation. It has also been suggested that thermotherapy causes a reduction in prostatic smooth muscle cell contractility or a long-term α-blockade [14, 15]. As was observed in α_1-adrenoceptor antagonist treatment, reduced

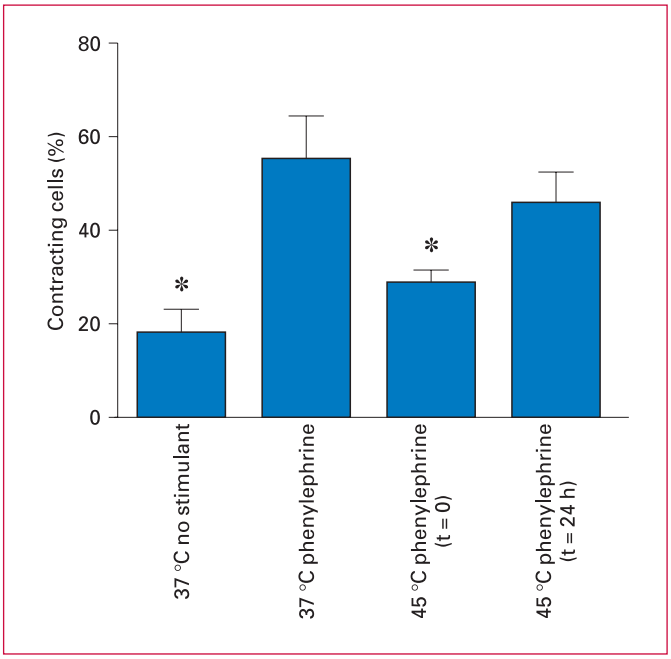

Fig. 3. Effect of thermotherapy on contraction rate of prostatic stromal cells. The histogram presents mean values and SD of 23 independent experiments with 10 cell strains. *$p < 0.05$ versus 37°C phenylephrine.

smooth muscle cell contractility may improve the symptoms of bladder outlet obstruction. There is yet another hypothesis suggesting involvement of neural elements [15, 16]. Histological studies showed severe thermal damage to intraprostatic nerve fibers caused by TUNA. Denervation of sympathetic and sensory nerves could explain the clinical improvement observed after thermotherapy.

In the present study, we have investigated the effects of heat exposure on the viability and contractility of prostatic stromal cells. Temperature mapping studies have shown that high-energy thermotherapies induce temperatures of 45–80 °C in the prostatic tissue [17–19]. For our study temperatures of 45 and 50 °C were chosen; this corresponds to the temperatures used in microwave therapy. At 50 °C, all cells instantly became necrotic. Therefore, temperatures higher than 50 °C were not applied in this study. Histologic examination of human prostatic tissue subjected to microwave therapy demonstrated that smooth muscle cells are more sensitive to elevations of temperature than other prostatic tissues (e.g. glandular cells) [4, 20]. Clinical data have shown a positive correlation between the stromal-epithelial cell ratio and the outcome of thermotherapy [21]. Consequently, prostates with a higher stromal cell content show a better response to thermotherapy. In control experiments, we were able to show that genital skin fibroblasts have a higher resistance to heat exposure than prostatic myofibroblasts. At a temperature of 50 °C about 30% of the fibroblasts survived 1 h. This possibly indicates that prostatic smooth muscle cells are less resistant to heat than other cellular components of the prostate such as fibroblasts and epithelial cells. The latter are likely to survive heat exposure and replace the necrotic smooth muscle cells. Hence, prostatic contractility appears to decrease without a reduction in prostatic volume, thus leading to an improvement in bladder outlet obstruction. This is in line with histopathologic findings in prostates following thermotherapy, which revealed infiltration of stromal fibroblasts into the area of necrosis leading to tissue revitalization [17].

At temperatures of 45 °C and less all cells survived. These findings correspond with results of animal and clinical studies, which yielded a temperature threshold of 45.7 °C for the induction of tissue necrosis [4, 17]. Successful cell passages following heat exposure suggest that no delayed cell damage was induced. At 45 °C, the contraction rate decreased only for a short period of time following heat application, but increased to nearly previous levels within 24 h. Further cell passages showed unchanged cell contractility. This clearly demonstrates that heat application has only a limited effect on the contractility of surviving smooth muscle cells. Furthermore, the results indicate that a temperature of 45 °C can hardly be expected to be effective in the treatment of BPH. These findings are in line with the results of clinical studies which did not yield any therapeutic effects of hyperthermia using temperatures of 42–43 °C [22]. However, it remains to be seen whether the findings of the present in vitro study actually reflect the in vivo situation. It may be speculated that cells in a three-dimensional organ are less sensitive to elevations of temperature than two-dimensional cell layer. Tissue impedance and tissue perfusion for example might protect the cells from damage by heat exposure, reducing effects of thermotherapies in vivo. Under in vivo conditions, thermotherapies might induce apoptotic rather than necrotic changes in some smooth muscle cells leading to delayed cell death. This could explain delayed improvement in lower urinary tract symptoms after microwave therapy, which is accelerated by neoadjuvant and adjuvant α-blockade [23]. Furthermore, heat application by TUMT or TUNA might provide additional specific effects as compared to heating in an incubation chamber.

Conclusions

With the help of video microscopy, it is possible to perform in vitro studies on the effects of thermotherapy on prostatic stromal cell contractility and viability. The results demonstrate that at temperatures of at least 50 °C, heat application acts primarily by inducing necrosis of contractile prostatic cells. At lower temperatures, however, the contractility of the surviving cells remains unchanged. Our data indicate that thermal therapy of BPH is probably only successful if temperatures in excess of 50 °C are achieved and maintained.

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