In Vivo Effects of High-Intensity Ultrasound on Prostatic Adenocarcinoma Dunning R3327

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ABSTRACT

High-intensity ultrasound has been used to treat Dunning R3327 prostatic adenocarcinoma implanted s.c. in Fischer Copenhagen rats. Focused ultrasound was generated with a 1-MHz transducer and energy was provided by a 7.5-kW power amplifier. Seventy-four rats were treated using two different sublines of Dunning tumor. Study 1 dealt with 49 rats with the Mat-Ly-Lu subline, treated with acoustic intensities ranging from 300 to 2750 W/cm². Of the 49 rats in Study 1, 30 had complete tumor necrosis and 19 had no effect; of the 30 who had complete local tumor necrosis, 14 had local relapse, 9 had distance metastases to lung and nodes without local occurrence, and 7 remained free of tumor and were still alive 12 months after treatment. In Study 2, 25 rats with AT2 subline were treated with an intensity of 820 W/cm². Similarly for Study 2, there was complete local tumor necrosis in 24 of 25 animals, with local regrowth in 7 of 24 and no recurrence of metastasis in the remaining 16 after a follow-up of 3 months. These results suggested that high-intensity focused ultrasound could be useful for the treatment of small localized canceous tumors such as low-grade prostatic carcinoma.

INTRODUCTION

Prostatic cancer is one of the most frequent malignant diseases in humans and no satisfactory treatment of localized tumor is available to patients older than 70 years. Consequently, new therapeutic options are urgently needed to extend to unqualified patients the potential benefits of surgery. These new options should ideally be noninvasive and capable of extracorporeal and selective destruction of tissue.

High-intensity ultrasound is a good candidate for this new therapeutic option because it is known to induce tissue lesions in vivo by sharply focusing an ultrasound beam on a specific target area within tissue. For a given amount of energy, tissue destruction may involve two distinct physical effects depending on acoustic intensity: (a) a thermal effect which is obtained by using long periods of exposure and low ultrasonic intensities; and (b) cavitation which is obtained with very high peak intensities.

This technique has been used experimentally for the destruction of small volumes in the brain (1) and in the eye for the treatment of glaucoma (2). It was shown that tissue lesions caused by a thermal effect (3) occurred at the focus of the ultrasound transducer, when acoustic energy was delivered with moderate intensity (less than 500 W/cm²) and the time of exposure was greater than 1 s. When acoustic energy was delivered with high-intensity ultrasound (greater than 3000 W/cm²) and a short exposure time, tissue lesions linked to cavitation occurred (4).

Thus we developed a device for extracorporeal-focused ultrasound tumor destruction. This device had previously been tested on healthy rat kidneys in vivo and was also capable of creating a sharply delineated, deep, and "trackless" tissue necrosis in vivo in the normal canine kidney (5). Recently, lesions of localized tissue necrosis in cat liver (6) and in pig kidney (7) in vivo were obtained by using high-intensity ultrasound. However, most studies have been performed on normal tissue while there have been few published reports on the treatment of experimental tumor (8). Subsequently, in order to prove the efficacy of high-intensity ultrasound in the treatment of malignant tumors, the authors initiated experimental studies with Dunning prostate adenocarcinoma R 3327 (Mat-Ly-Lu and AT2 sublines) implanted s.c. in rats. This experimental study had two goals: to both destroy and cure this metastatic tumor. The results of these studies are presented.

MATERIALS AND METHODS

Animals and Tumor Model

Fischer Copenhagen rats, obtained from Harlan Olac (Great Britain), were housed in our laboratory. Only male rats, 10 to 12 weeks old, were used in all experiments.

R 3327 Dunning prostatic adenocarcinoma sublines (Mat-Ly-Lu and AT2) were a gift of Dr. Schalken (Research Department of Pr. Debruyne, Nijmegen, the Netherlands). Cells were kept in liquid nitrogen. For our experiments, cellular samples of the same pool were warmed and cultured under standard conditions. A rat was given an abdominal s.c. injection of 2 x 10⁶ cells and after 8 days, a solid tumor was obtained, excised, and minced; 20-µg pieces were then implanted in the experimental animals. Under these conditions, without treatment, mortality due to tumor normally occurs within 3 weeks after implantation with the Mat-Ly-Lu subline. The pattern is drastic, with enormous lung and lymph node metastasis and a primary tumor as large as 20 to 40 cm³. For the AT2 subline, 7 to 8 weeks are necessary to induce death. Metastases, when present, are few, are inconsistent, and involve only lymph nodes but never the lungs (Table 1). Although metastasis occurred, the death rate is delayed with this AT2 subline and survival curves can be established.

Material

As shown in Fig. 1, the prototype device used for treatment, Ablatherm (Technomed, Bron, France), consisted of 3 integrated components: (a) an ultrasound treatment system located in a tank filled with de-gassed and deionized water; (b) an imaging system consisting of an ultrasound scanner coupled with a stereotaxic localizing arm; and (c) a computer which controlled the firing sequence and the movements of the firing head through a 3-dimensional positioning system. The firing head consisted of a highly focused 10-cm-diameter 1-MHz piezoceramic transducer, which produced in water an acoustic beam the focus of which was 2 x 10 mm at 10-cm focal distance. A linear power amplifier capable of delivering 7.5 kW at a frequency of 1 MHz was used for insonation. The computer controlled the bursts produced by a signal generator (HP 8116 A) resulting in a complete 18- to 25-s cycle (3-10 s on and 15 s off).

Treatment Session

Under general anesthesia, the animal was placed on a specially designed Plexiglas gantry with an opening below the abdominal area for...
HIGH-INTENSITY ULTRASONIC THERMAL DESTRUCTION OF DUNNING TUMORS IN RATS

Table 1 In vivo biological characteristics of Dunning R 3327 rat prostatic tumor sublines used in the study (after Isaacs)

<table>
<thead>
<tr>
<th>Subline</th>
<th>Mat-Ly-Lu</th>
<th>AT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td>Anaplastic</td>
<td>Anaplastic</td>
</tr>
<tr>
<td>Growth rate (doubling time, days)</td>
<td>1.5 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Androgen responsive</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Metastatic potential (lymph nodes or lungs)</td>
<td>High (lymph nodes and lungs)</td>
<td>Low to moderate (lymph nodes)</td>
</tr>
<tr>
<td>Host survival (days)</td>
<td>26 ± 1</td>
<td>63 ± 3</td>
</tr>
</tbody>
</table>

Fig. 1. Therapy system used to produce focal lesions by high-intensity ultrasound.

The penetration of ultrasound (Fig. 2). The tumor was immobilized with a silicone ring fastened to the gantry. This gantry was then mounted on the x-y-z positioning system in order to grossly position the animal over the generator at the approximate focal distance. After the tank was filled with warm (35°C) degassed and deionized water, an initial localization of the target area with a 5-MHz ultrasound probe was performed. Using the locating arm of the Ablatherm prototype, the coordinates of the target volume were sent to the instrument computer which controlled the movements of the ultrasound therapeutic transducer. Programming by the computer software produces a number of shots, according to a specific volume the dimensions of which correspond to an area extended 2 mm beyond the visible tumor margin. Once all the parameters were entered (i.e., x, y, and z dimensions of the target), diameter of the elementary lesions induced by one burst, duration of the burst, and interval between bursts, the treatment session was started.

Choice of Treatment Parameters

Previous work in the normal rat kidney in vivo (5) specifically studied optimal tissue destruction, taking into account the ultrasound intensity, the length of bursts, and the interval between bursts. These results were used in a preliminary study (9) to define optimal parameters for Dunning tumor destruction in vivo. These parameters were found to be in the specific range of 300 to 2750 W/cm² for the intensity and 3 to 10 s for burst duration, inasmuch as intensities from 3000 to 8000 W/cm² produce only cavitation effects (9).

Experimental Setup

Two studies were successively performed.

Study 1. The first experiment was performed with the Mat-Ly-Lu subline to confirm parameters obtained in the previous study (9). Five days after implantation, 85 animals bearing an average 320-mm³ tumor were randomly divided in 2 groups. Group 1 (n = 49) received a focused ultrasound treatment with intensities ranging from 300 to 2750 W/cm² and insonation times ranging from 3 to 10 s (Table 2). Group 2 (n = 36), transplanted under the same conditions, was used as control.

Tumor volume was calculated by measuring the three spatial dimensions of the tumor with an electronic digital caliper, according to the ellipsoid formula. Tumor growth curves of insonated animals were determined and compared to those of the control group. All animals,
still alive on the 21th day after therapy but with evidence of local regrowth of tumor or metastasis, were sacrificed and autopsies were performed for histopathological analysis. Animals with no evidence of tumor regrowth or metastasis were kept for a long-term follow-up. For ethical reasons, control rats who were still alive on the 26th day after tumor implantation were sacrificed.

Study 2. The second experiment was performed with the AT2 sub-line which was used because of the less aggressive nature of the cells and the lower metastatic potential.

Fifty rats, given injections of 20-mg solid tumor pieces at the same time, were randomly divided into 2 groups of 25 animals each. Then, each group was randomly divided into 5 subgroups of 5 animals each. Subgroups of Group 1 received ultrasound therapy and subgroups of Group 2 (the control group) did not. On the seventh day after implantation, when tumor volume reached roughly 320 mm³, animals in each subgroup were anesthetized and placed in the Plexiglas gantry with the tumor insonated for 9 s at an intensity of 820 W/cm². Animals in control subgroups were submitted to the same experimental procedure excluding insonation.

After treatment, tumor growth curves, survival curves, and disease-free intervals were studied using the following statistical analysis. (a) Tumor volume measurements were performed, at a predetermined time (5, 9, 15, 20, and 26 days) after treatment, with the rats of two subgroups which were randomly selected, once and for all, in each group. Growth curves were presented as an average of tumor volumes for each subgroup. (b) The mean and SD of tumor volume of all rats in each group were calculated at 15-day intervals (immediately, 15 and 30 days, respectively) after treatment and compared using a Wilcoxon test. (c) The survival curves were made with the Kaplan-Meier estimate method and the differences were analyzed for significance using the log rank method. Rats in the control group and uncured animals in Group 1 were observed until they died of progressive tumor growth or metastasis. Autopsy and histopathological study of the primary site were performed to confirm macroscopic observations. Other animals were kept indefinitely for long-term follow-up.

### RESULTS

Study 1. Most of the 36 animals in the control group with Mat-Ly-Lu subline died of tumor growth and others were sacrificed. Fig. 3 shows the normal tumor growth curve of this group where an average 40-cm³ volume could be observed 21 day after implantation. Among the 49 animals that received treatment, no effect or a slight decrease in growth occurred in 19 animals (39%). Total tumor destruction was achieved in 30 rats (61%) for which 3 evolutions could be observed. (a) An initial complete and immediate tumor necrosis occurred in 14 animals (29%) but a local regrowth of tumor from the periphery appeared after 1 week. In these animals, we observed a local enlargement of the primary tumor and a delayed growth rate which progressively became the same as for controls. (b) A complete and immediate tumor necrosis with no evidence of local tumor regrowth occurred in nine animals (18%). There was no evidence of tumor at the treatment site but impressive axillary or inguinal lymph node metastasis appeared within 3 weeks. All nine animals were sacrificed. Autopsies showed numerous lung metastases and histopathological examination of the primary tumor site confirmed the healing of the tissue (skin and muscle). (c) Seven animals (14%) appeared to be cured without any local relapse or metastasis. They were still alive 12 months after treatment. One rat was sacrificed at day 80 for histopathological analysis of lungs, lymph nodes, and tumor implantation area. No evidence of malignant disease was observed in this examination.

Overall, the variation of combinations of ultrasound intensity and insonation time (Table 1) did not produce significant differences in the subgroup results. Therefore, data were treated as if they were only one group. This finding is consistent with what we expected with a narrow range of intensities and durations.

Study 2. A complete tumor necrosis occurred in 24 animals (96%) in the group with AT2 subline receiving high-intensity ultrasound therapy. One rat died prematurely on the 6th day after treatment due to infection of the necrotic tumor area. The tumor of one rat, outside the ultrasound beam area, showed no effect or change in growth rate. A local regrowth of tumor from the periphery was identified in seven animals (28%). Sixteen rats were still alive on the 115th day after treatment without any pathological evidence of tumor regrowth or metastasis (64%). Tumor growth curves are shown in Fig. 4. As outlined in Table

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**Table 2 Parameters of one shot and numbers of animals used in Study 1 for treatment of rats with Mat-Ly-Lu subline of Dunning R 3327**

<table>
<thead>
<tr>
<th>Ultrasound intensity (W/cm²)</th>
<th>Burst duration (s)</th>
<th>No. of rats treated (n = 49)</th>
<th>No. of controls used (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>1000</td>
<td>9</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>1500</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2000</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2750</td>
<td>3</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

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**Fig. 3. Tumor growth curve of control group with Mat-Ly-Lu Dunning tumor.**

- □, mean value of 36 measurements.

**Fig. 4. Tumor growth curves of 50 rats, with AT2 subline of Dunning tumor.**

- □, mean values of each subgroup in the control group; ×, mean values of each subgroup in the group receiving high-intensity ultrasound therapy. Bars (SD) in each subgroup.
3, there was a significant difference ($Z = 5.75; P < 0.0001$) in the mean tumor volume on the 15th and 30th days between the ultrasound treatment group and the control group. Additionally, there was a significant difference ($P < 0.0001$) in survival curves between the two groups as shown in Table 4 and Fig. 5. Finally, Fig. 6 shows disease-free interval for the treated group.

All rats in the control group died of progressive tumor growth. Of these, seven developed lymph node metastasis (28%). In the treatment group, four animals (16%) also presented lymph node metastasis at autopsy. This occurred, however, only in animals with local regrowth of tumor.

**DISCUSSION**

The efficacy of focused ultrasound on prostatic Dunning adenocarcinoma is much higher than the efficacy of other treatments reported in the literature. The Dunning R 3327 is a radio resistant tumor as shown by Mador (10) in that external insonation stopped tumor growth but could not destroy it. Histopathological studies showed viable cells in the treated area after insonation. Mador also showed that cytotoxic drugs (cisplatin, vincristine and etoposide) induced only a transitory halt of tumor growth. Many authors such as Russo (11) and Oosterhof (12) have studied the impact of shock waves on this tumor line. For Russo, a transitory decrease in the growth was obtained for 5 days while no impact on growth with shock waves was shown by Oosterhof and Debruyne in the treatment of R 3327 AT3 tumors (subline very close to Mat-Ly-Lu). They suggested that this experimental model might be inappropriate for in vivo studies. In Study 1 with Mat-Ly-Lu subline, we think that the results in the treated group of no effect (39%) and local relapse (14%) can be explained either by an inadequate ultrasound scanning of the target volume (the treated area did not include all the tumor volume) and/or by an insufficient ultrasound dose delivered in some series (the ultrasound intensities varied between 300 and 2750 W/cm² while the exposure times varied between 3 and 10 s). Otherwise we think that the metastatic diffusion without local recurrence, observed in 18% of treated rats, was induced by early metastatic swarming prior to treatment.

Finally, in Study 1, while only 14% of the treated rats were cured, this result was better than other published treatments. We conclude that, under certain conditions, focused ultrasound was capable of destroying the experimental Dunning prostatic adenocarcinoma of the Mat-Ly-Lu subline and was able to cure this experimental malignant disease without any adjuvant treatment.

In Study 2 with subline AT2, the enhancement of results can be explained either by the improved localization system or by an extended scanning of the tumor (the treated area was larger than the tumor volume) or by an improved selection of ultrasonic dose (820 W/cm² by 9 s for each shot). However, although a large number of treated animals were completely cured, we observed in some animals a local tumor regrowth which can be explained by the inefficiency of some shots in the firing sequence due to the presence of cavitation effects. We showed in a previous study (13) that at an intensity greater than 3000 W/cm² cavitation inhibited thermal effects. At an intensity of 820 W/cm² and exposure time of 9 s, heating was certainly the dominant mechanism for tissue destruction, but it was not fully understood whether nonthermal effects, such as cavitation, induced side effects. Temperature measurements were obtained in one experiment in Study 1, using a 50-μm thermocouple placed under ultrasound control in order to define the temperature rise at the focus of the ultrasound beam. Internal temperature reached 78°C in 3 s with an intensity of 2750 W/cm². Although

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**Table 3** In Study 2: comparison of mean tumor volumes of all rats in Groups 1 and 2, immediately and on the 15th and 30th days after treatment by high-intensity ultrasound

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Controls (mm³)</th>
<th>Treated (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>281 ± 18</td>
<td>331 ± 13</td>
</tr>
<tr>
<td>15</td>
<td>4753 ± 318</td>
<td>325 ± 270</td>
</tr>
<tr>
<td>30</td>
<td>18541 ± 1179</td>
<td>1978 ± 903</td>
</tr>
</tbody>
</table>

* Number of rats in each group, 25.
* Mean ± SE.

**Table 4** Survival rate comparison in Study 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Controls</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Free of tumor</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>No. of deaths</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Expected events</td>
<td>11.22</td>
<td>22.78</td>
</tr>
<tr>
<td>Ratio (observed/expected)</td>
<td>2.23</td>
<td>0.40</td>
</tr>
<tr>
<td>Median (days)</td>
<td>62</td>
<td>113+</td>
</tr>
<tr>
<td>Expectancy (days)</td>
<td>61</td>
<td>94+</td>
</tr>
</tbody>
</table>

Chi²: Homogeneity 31.0229 ($P < 0.0001$) Log rank
a higher measurement could be expected, these measurements were in good correlation with the histological findings of cellular necrosis and tissue lesions.

A potential risk of focused ultrasound therapy was the possible enhancement of metastatic development if the radiation strength produced by the ultrasound beam "pushed" tumor cells through damaged blood vessels into the circulation. Fry and Johnson (14) have observed a higher rate of secondary tumor development after ultrasound therapy. However, in our study on AT2 subline the metastatic rate observed in nontreated rats (28%) was higher than the metastatic rate observed in treated rats (16%). In animals receiving treatment, no metastasis occurred with local control of the tumor while metastatic evolution was observed only in rats with local tumor regrowth. Therefore, it seems that high intensity ultrasound did not increase metastatic risk. However, further research is necessary to conclude definitively.

The results of this study suggested that high-intensity ultrasound therapy was capable of destroying the Mat-Ly-Lu and AT2 sublines of the Dunning R 3327 prostatic adenocarcinoma and induced a complete cure of this malignant disease in 14 and 64% of cases, respectively.

In the future, we believe that prostatic tumors in humans are ideally suited to be treated by focused ultrasound for the following reasons: (a) prostatic tissue and the tumor are relatively homogeneous in acoustic impedance; and (b) the prostate can be easily treated with ultrasound via an endorectal route. To support this view, an experimental study on human prostatic adenocarcinoma, implanted in nude mice, has been started to study the effects of high-intensity ultrasound on this tumor.

ACKNOWLEDGMENTS

The authors greatly appreciate the help of E. Blanc from Technomed International for his technical assistance.

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