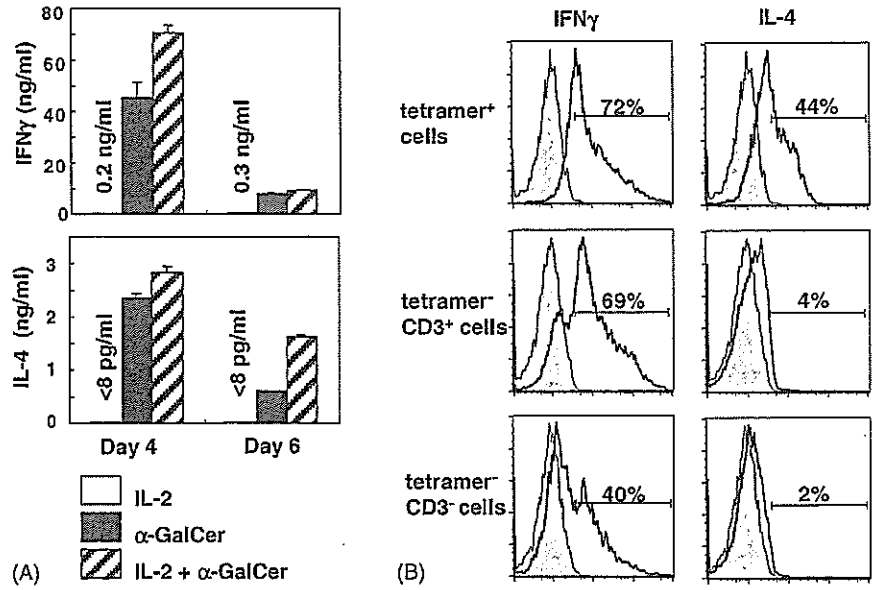

Fig. 3. Cytokine production profile of Vα14i NKT cells, NK cells, and NK1.1$^+$ T cells after culture. (A) Spleen cells (7×10^6) from C57BL/6 mice were cultured with 50 ng/ml α-GalCer, α-GalCer plus 100 U/ml IL-2, or IL-2 for 4 and 6 days. IFNγ and IL-4 in the supernatants were measured by ELISA. Data are representative of three independent experiments. (B) Intracellular cytokine staining for IFNγ and IL-4 in spleen cells cultured with α-GalCer and IL-2 for 4 days. The cultured cells were stimulated with PMA and ionomycine for 2 h. Then, the cells were stained with CD1d/α-GalCer tetramer, anti-CD3 mAb, and anti-IL-4, IFNγ, or isotype control mAb and analyzed by flow cytometry. Histogram panels are on CD1d/α-GalCer tetramer$^+$ CD3$^+$ cells (Vα14i NKT cells), CD1d/α-GalCer tetramer$^-$ CD3$^+$ cells (including NK1.1$^+$ T cells), or CD1d/α-GalCer tetramer$^-$ CD3$^-$ cells (including NK cells). Closed histograms indicate isotype controls. The fluorescence profiles are representative of three independent experiments.

Next, we examined the ability of the adoptively transferred, in vitro-expanded Vα14i NKT cells, to secrete IL-4 and IFNγ after administration of α-GalCer. Seven days after cell transfer, the mice were injected with 2 μg of α-GalCer.

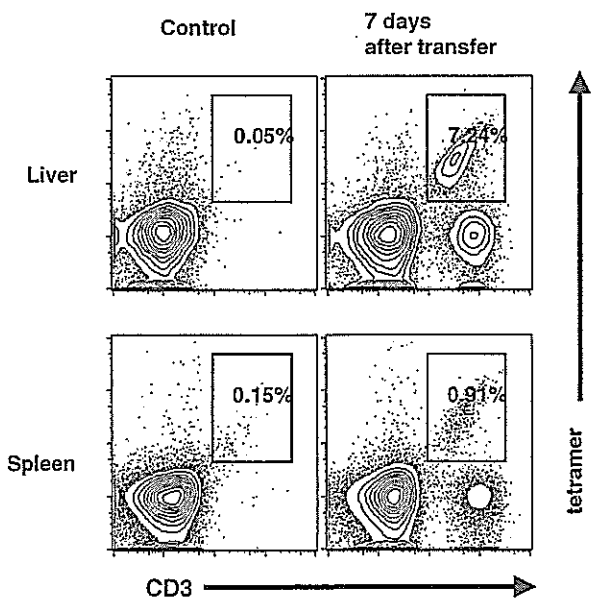

Fig. 4. Migration of in vitro-expanded Vα14i NKT cells after adoptive transfer. Spleen cells from BALB/c mice were cultured with α-GalCer plus 100 U/ml IL-2 for 4 days. The cultured cells (2×10^7) were injected into C.B-17/Icr SCID mice. Recipient mice were killed after 7 days, and the presence of transferred Vα14i NKT cells in the liver and spleen was determined by flow cytometry. The fluorescence profiles are representative of three independent experiments.

The serum levels of IL-4 and IFNγ were analyzed by ELISA. Four hours after the α-GalCer injection, IL-4 and IFNγ were detected in the serum of mice that had received cultured cells (Fig. 5A). One hour after α-GalCer administration, intracellular cytokine staining for CD1d/α-GalCer tetramer$^+$ T cells in the spleen revealed that intracellular IL-4 and IFNγ were detected in 50 and 30% of CD1d/α-GalCer tetramer$^+$ T cells, respectively (Fig. 5B). These results indicate that the expanded Vα14i NKT cells re-exposed to α-GalCer retain the ability to produce IL-4 and IFNγ after adoptive transfer.

It has been reported that increased IFNγ levels in the serum of normal mice 10–16 h after α-GalCer injection were due to IFNγ production by NK cells [11]. However, 10 h after the α-GalCer injection, the IFNγ level was decreased in the mice that had previously received the cultured cells. Therefore, the in vitro-expanded Vα14i NKT cells could not induce NK cell IFNγ production in vivo. Previous reports have demonstrated that diminished IFNγ levels in the serum of α-GalCer-primed mice were caused by a failure of NK cell IFNγ production after α-GalCer re-injection [27]. Thus, the in vitro-expanded Vα14i NKT cells might be similar to primed Vα14i NKT cells.

4. Discussion

NKT cells play an important role in various immune responses, including autoimmunity and tumor immunity [1–3]. The administration of α-GalCer, a specific ligand

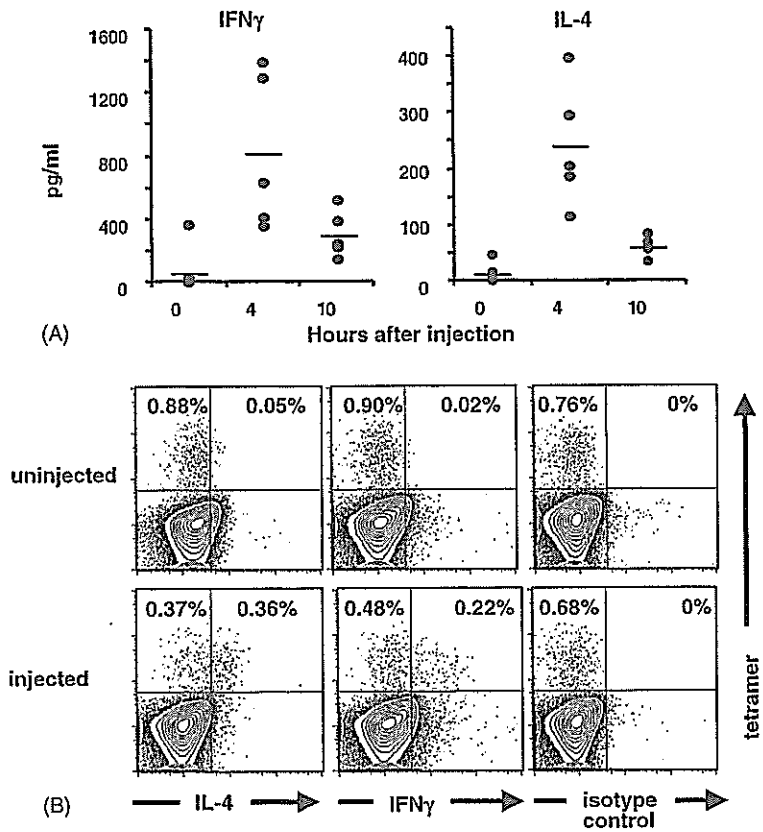

Fig. 5. IFN-γ and IL-4 production of in vitro-expanded Vα14i NKT cells after adoptive transfer. Spleen cells from BALB/c mice were cultured with α-GalCer plus 100 U/ml IL-2 for 4 days. The cultured cells (2×10^7) were injected into C.B-17/Icr SCID mice. (A) Serum IFN-γ and IL-4 levels. Seven days after the cells were injected, the serum cytokine levels were analyzed 0, 4, and 10 h after i.p. injection of α-GalCer. Data were obtained from 5 to 7 mice. (B) Intracellular cytokine staining of splenocytes 2 h after i.p. injection of α-GalCer (2 μg) in mice injected with cultured cells (2×10^7) 7 days earlier. Cells were stained with CD1d/α-GalCer tetramer and anti-IL-4, IFN-γ, or isotype control mAb. Stained cells were analyzed by flow cytometry. The fluorescence profiles are representative of three independent experiments.

for Vα14i NKT cells, prevents tumor metastasis [9,10] and autoimmune disease [28–30]. Moreover, the adoptive transfer of NKT cells in mice prevents type I autoimmune diabetes [31] and tumor metastasis [12,32]. These studies suggest several possible therapeutic applications for adoptive immune therapy with NKT cells. However, it is apparent that the frequency of NKT cells is very low in human blood. Therefore, in vitro NKT cell expansion is required for adoptive immunotherapy with these cells. In this study, we found that in vitro-expanded Vα14i NKT cells are able to migrate into liver and spleen, and produce cytokines after adoptive transfer.

Human $V\alpha24^+V\beta11^+$ T cells in peripheral blood mononuclear cells expand in vitro using α-GalCer and IL-2, IL-7, or IL-15 [13–19], and mouse Vα14i NKT cells also proliferate in the presence of α-GalCer in vitro [1–3]. However, the function and phenotype of in vitro-expanded Vα14i NKT cells have not been well characterized because there is no appropriate marker to identify these cells. In previous studies, NKT cells have been identified as $NK1.1^+$ T cells. However, some Vα14i NKT cells do not express NK1.1 [25,26], and Vα14i NKT cells lose or down-regulate the expression of NK1.1 in vivo after stimulation [33,34]. Therefore, the NK1.1 marker is not expressed on Vα14i NKT cells after stimulation. We could detect in vitro-expanded Vα14i NKT cells by CD1d/α-GalCer tetramer. However, an issue with CD1d/α-GalCer tetramer staining is that the surface expression of Vα14i NKT cells is also down-regulated at 8–12 h after α-GalCer-stimulation [33,34]. Although their TCR expression was recovered to normal levels at 24–48 h [33,34], it is not an issue is whether the numbers of in vitro-expanded Vα14i NKT cells (at 4 and 6 days after culture) is an underestimate of the actual number. These in vitro-expanded Vα14i NKT cells in the presence of α-GalCer do not express NK1.1. It has been reported that $NK1.1^-$ CD1d/α-GalCer $tetramer^+$ T cells exist in normal mice and that some of these cells are immature NKT cells that have recently emigrated from the thymus [35,36]. However, recent studies have shown that expanded $NK1.1^-$ Vα14i NKT cells originate from $NK1.1^+$ Vα14i NKT cells that down-regulate their surface NK1.1 expression [33,34]. We considered two possibilities for the origin of in vitro-expanded Vα14i NKT cells: expansion of NK1.1 down-regulated NKT cells and/or expansion of $NK1.1^-$ precursor NKT cells. We observed that some Vα14i NKT cells expanded when $NK1.1^-$ spleen cells were cultured (data not shown). Therefore, we concluded that both

NK1.1$^+$ and NK1.1$^-$ NKT cells expand after in vitro α-GalCer-stimulation.

Previous studies demonstrated that mouse and human invariant NKT cells could produce both Th1 and Th2 cytokines [1–3]. Furthermore, it was reported that adult Vα24$^+$ Vβ11$^+$ NKT cells did not polarize into Th1 or Th2 after expansion [37]. However, NKT cells display polarization induced by type 1 or 2 dendritic cells [19]. Th1 or Th2 polarization of NKT cells is believed to be influenced by culture environment, such as the type of dendritic cell. We showed that in vitro-expanded Vα14i NKT cells continuously produced both IL-4 and IFNγ and did not polarize into Th1- or Th2-type. By contrast, a previous study has demonstrated that the robust expanded Vα14i NKT cells (after α-GalCer administration) continue to produce IFNγ in vivo [34]. This suggests that in vivo-expanded Vα14i NKT cells favor Th1 polarization. In contrast to in vivo-expanded Vα14i NKT cells, in vitro-expanded Vα14i NKT cells might remain continually activated and produce both IFNγ and IL-4 because they are continually exposed to α-GalCer in the culture conditions. Indeed, in the expansion phase of Vα14i NKT cells (after α-GalCer injection), these Vα14i NKT cells had the ability to secrete large amounts of both cytokines when re-injected with α-GalCer (Ikarashi et al., unpublished data).

In addition to the IFNγ production by in vitro-expanded Vα14i NKT cells, we showed that NK cells and NK1.1$^+$ T cells acquired the ability to produce IFNγ when cultured with α-GalCer and IL-2. IL-2 alone could induce the proliferation, but not IFNγ production, by NK cells and NK1.1$^+$ T cells in vitro. Our results indicate that α-GalCer-induced Vα14i NKT cell activation leads to IFNγ production of NK and NK1.1$^+$ T cells in vitro. Previous in vivo studies have demonstrated rapid cytokine production by Vα14i NKT cells in response to α-GalCer triggered activation and IFNγ production by NK cells [7,8], and bystander activation by conventional T cells and B cells [38,39]. Taken together, the mechanisms of NK cell activation by Vα14i NKT cells in vitro might be similar to the in vivo mechanisms.

Vα14i NKT cells have been known to regulate immune responses [1–3]. In fact, previous studies have shown that adoptive transfer of thymic NKT cells prevented type I diabetes in NOD mice in an IL-4- and IL-10-dependent manner (T helper 2) [31]. Furthermore, hepatic metastasis of B16 melanoma was prevented by adoptive transfer of IL-12-activated Vα14i NKT cells from Vα14 TCR transgenic mice [32]. These observations indicate that adoptive Vα14i NKT cell immunotherapy is useful for autoimmune diabetes and cancer. However, important questions remain as to whether in vitro-expanded Vα14i NKT cells can survive in the recipients and maintain the ability to produce IFNγ and IL-4 after transfer, similar to resident Vα14i NKT cells. A previous study demonstrated that fresh mouse Vα14i thymocytes proliferated and survived in an IL-15-dependent manner after adoptive transfer into lymphopenic mice [40]. We found that in vitro-expanded Vα14i NKT cells survived and dominantly migrated into the liver of lymphopenic mice. Furthermore, we revealed that in vitro-expanded Vα14i NKT cells 7 days after transfer could respond to α-GalCer and secrete IL-4 and IFNγ after administration of α-GalCer. However, IFNγ production patterns of lymphopenic mice transferred with in vitro-expanded Vα14i NKT cells after administration of α-GalCer were similar to those of α-GalCer-primed mice, as reported previously [27].

Previous studies have demonstrated that the antitumor effect of α-GalCer is mediated by Vα14i NKT cells [9–12] and that the IFNγ production by Vα14i NKT cells and the subsequent IFNγ production by NK cells are critical for α-GalCer to mediate antitumor activity [11,12]. Although it appears that α-GalCer-based immunotherapy is useful for cancer, treatment with α-GalCer has shown little therapeutic effect in patients with solid tumors [41]. For these reasons, it has been proposed that human Vα24i NKT cells from cancer patients have impaired proliferative responses to α-GalCer and have lost the ability to produce IFNγ [22,23]. We believe that adoptive Vα24i NKT cell therapy may be beneficial for cancer patients. Further studies are needed to clarify the mechanism and clinical applicability of in vitro-expanded Vα24i NKT cell therapy in cancer.

Acknowledgments

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TRANSRECTAL HIGH-INTENSITY FOCUSED ULTRASOUND IN THE TREATMENT OF LOCALIZED PROSTATE CANCER : A MULTICENTER STUDY

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We report a multicenter trial with transrectal high-intensity focused ultrasound (HIFU) in the treatment of localized prostate cancer. A total of 72 consecutive patients with stage T1c-2N0M0 prostate cancer were treated using the Sonablate 500™ HIFU device (Focus Surgery, Indianapolis, USA). Biochemical recurrence was defined according to the criteria recommended by the American Society for Therapeutic Radiology and Oncology Consensus Panel. The median age and prostate specific antigen (PSA) level were 72 years and 8.10 ng/ml, respectively. The median follow-up period for all patients was 14.0 months. Biochemical disease-free survival rates in all patients at 1 and 2 years were 78% and 76%, respectively. Biochemical disease-free survival rates in patients with stage T1c, T2a and T2b groups at 2 years were 89, 67% and 40% ($p=0.0817$). Biochemical disease-free survival rates in patients with Gleason scores of 2-4, 5-7 and 8-10 at 2 years were 88, 72% and 80% ($p=0.6539$). Biochemical disease-free survival rates in patients with serum PSA of less than 10 ng/ml and 10-20 ng/ml were 75% and 78% ($p=0.6152$). No viable tumor cells were noted in 68% of patients by postoperative prostate needle biopsy. Prostatic volume was decreased from 24.2 ml to 14.0 ml at 6 months after HIFU ($p<0.01$). No statistically significant differences were noted in International Prostate Symptom Score, maximum urinary flow rate and quality of life analysis with Functional Assessment of Cancer Therapy. HIFU therapy appears to be minimally invasive, efficacious and safe for patients with localized prostate cancer with pretreatment PSA levels less than 20 ng/ml.

(Hinyokika Kiyo 51 : 651-658, 2005)

Key words : Prostate cancer, High-intensity focused ultrasound, Minimally invasive surgery

INTRODUCTION

Prostate cancer is the most common malignancy in men and the second leading cause of death due to cancer in the United States[1]. Prostate cancer has been treated in various ways, depending on the severity of the condition, age of the patient, staging, Gleason score and serum prostate-specific antigen (PSA) level. Radical prostatectomy has long been regarded as appropriate therapy for patients with organ-confined prostate cancer. Despite excellent 5- to 10-year survival rates after radical prostatectomy for organ-confined disease, surgery is

associated with significant morbidity, including blood loss due to transfusion-related complications, erectile dysfunction in 30% to 70% of cases, and stress incontinence in up to 10% of patients[2,3]. In addition, surgical intervention is not typically considered for patients whose life expectancy is less than 10 years. Recently, a number of alternative less invasive treatments have been developed for patients with localized prostate cancer, either not appropriate for surgery or who do not want to risk the potential side effects of surgery. Three-dimensional conformal radiotherapy (3D-CRT), brachytherapy, intensity-modulated external beam radiotherapy, cryosurgical ablation of the prostate and laparoscopic radical prostatectomy have all been applied for the treatment of this group of patients[4-6]. However, in the event of treatment failure, these cannot be repeated and salvage radical prostatectomy is associated with a high morbidity rate[7].

High-intensity focused ultrasound (HIFU) delivers intense ultrasound energy with consequent heat destruction of tissue at a specific focal distance from the probe without damage to tissue in the path of the ultrasound beam[8]. HIFU non-invasively induces complete coagulative necrosis of a tumor without surgical exposure or insertion of instruments into the lesion. This advantage makes it one of the most attractive options for the localized treatment of tumors[9,10]. We report here a multicenter trial with 72 consecutive patients treated with HIFU for clinical stage T1c-2N0M0 localized prostate cancer.

PATIENTS AND METHODS

Inclusion and Exclusion Criteria

As a rule, the inclusion criteria for treatment were patients with biopsy proven and untreated stage T1c-2N0M0 localized prostate cancer[11]. Age, serum PSA levels, prostatic volume and WHO performance status should be less than 80 yrs, 20 ng/ml, treatable with a 4.0 focal length probe which means a prostatic volume less than 50 ml and 0-1. Patients with urethral stricture, anal stricture, bleeding tendency, renal dysfunction with serum Cr more than 2.0 mg/dl, hydronephrosis, larger than 5 mm calcifications in the prostate, uncontrolled diabetes mellitus, hypertension, angina, history of cardiac infarction or other malignant diseases were excluded from the study. None of the patients were receiving neoadjuvant hormonal and/or chemotherapy before HIFU. All patients were fully informed of the details of this treatment and gave written consent preoperatively.

HIFU Eqipment

For this study, we used the Sonablate 500™ (Focus Surgery, Indianapolis, IN, USA) HIFU machine. This treatment module includes the ultrasound power generator, transrectal probes, the probe positioning system, and a continuous cooling system (Fig. 1). The

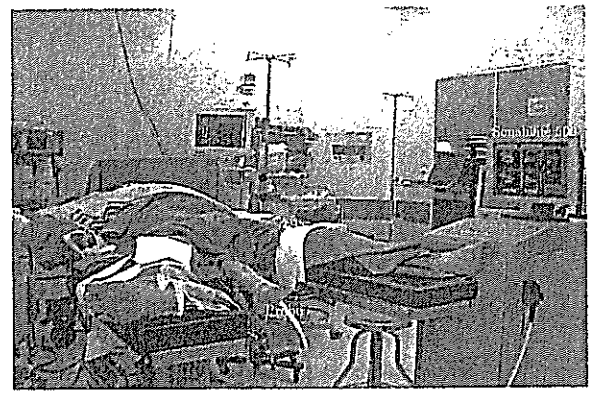

Fig. 1. The Sonablate-500™ type device consists of an operator's console, imaging monitor, transrectal probe and an automatic continuous cooling system.

transrectal HIFU probes use proprietary transducer technology with low-energy ultrasound (4 MHz) for imaging of the prostate and for the delivery of high-energy ablative pulses (site intensity, 1,300-2,200 W/cm^2). The single piezoelectric crystal alternates between high-energy power for ablative (3 sec) and low-energy for ultrasound imaging (6 sec)[10].

Prior to beginning the treatment, the operator uses longitudinal and transverse sonograms to obtain an image of the prostate and selects the prostate tissue volume to be ablated by a set of cursors on these images. The probe houses a computer-controlled positioning system that directs each ablative pulse to the targeted region of the prostate. Each discrete high-energy focused ultrasonic pulse ablates a volume of $3 \times 3 \times 10$ mm^3 of tissue[10]. The total acoustic power is initially set at 24 W and 37 W for 3.0 and 4.0 cm focal length probes, respectively. The individual focal lesion produces almost instantaneous coagulative necrosis of the tissue due to a temperature rise of 80° to 98°C in the focal zone[8]. Under computer control, the ultrasound beam is steered mechanically to produce consecutive lesions in a manner such that all focal lesions overlap

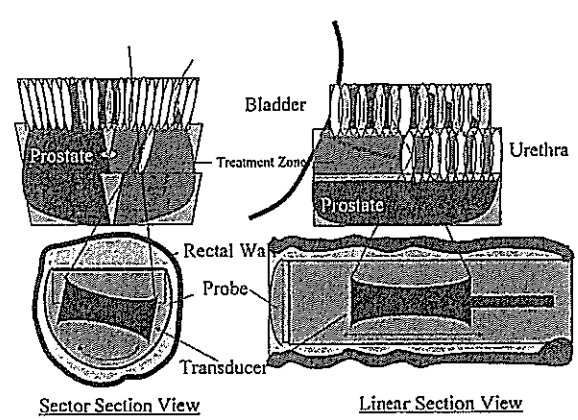

Fig. 2. The computer-controlled transducer ablates the entire prostate tissue. Focal lesions are overlapped in linear rows (left) at each of the lateral sector positions (right) to create a volume lesion.

laterally and longitudinally to ensure necrosis of the entire targeted prostate volume (Fig. 2). An automatic cooling device is used during treatment to maintain a constant baseline temperature of less than 18°C in the transrectal probe that helps to prevent thermal injury of the rectal mucosa.

HIFU Procedure

All patients were anesthetized by general, epidural, spinal or intravenous anesthesia, and were placed in a supine and open leg position. A condom was placed over the probe and degassed water was used to inflate the condom that was covered with ultrasound gel for close coupling of the ultrasound probe to the rectal wall, and the probe was inserted manually into the rectum. The probe was fixed in position by an articulating arm attached to the operating table. After selection of the treatment region of the prostate from the verumontanum to the bladder neck, the treatment was started. Transrectal probes with focal lengths of 3.0 and 4.0 cm were used according to the size of the prostate as determined by transrectal ultrasound (TRUS), with larger glands requiring longer focal lengths. The treatment continued layer by layer (10 mm thickness) from the apex to the base (Fig. 2). Usually, three successive target areas (anterior, mid-part and base) were defined to treat the whole prostate. After treatment was completed, a transurethral balloon catheter was inserted into the bladder[10].

Clinical Follow-up and Definition of Outcome

Patient status and treatment-related complications were followed up by all available means, including periodic patient visits and self-administered questionnaires dealing with urinary continence and erectile function using Functional Assessment of Cancer Therapy (FACT) questionnaire. Urinary symptoms and urinary flow rate analysis were performed using International Prostate Symptom Score (I-PSS) index and urowflowmetry[12,13]. Serum PSA was assayed every 1 to 6 months during follow-up. A postoperative prostate needle biopsy under TRUS was performed on all patients at 6 months. The American Society for Therapeutic Radiology and Oncology (ASTRO) consensus definition, i.e., three consecutive increases in post treatment PSA after a nadir has been achieved, was used to define biochemical failure[14]. The time to biochemical failure was defined as midway between the post treatment PSA nadir and the first of three consecutive PSA increases. None of the patients received androgen deprivation after HIFU or other anticancer therapy before documentation of a biochemical recurrence. HIFU related complications were defined by Japanese version of National Cancer Institute-Common Toxicity Criteria version 2.0[15].

Statistical Analyses

All statistical analyses were performed by the Department Statistics in Indiana University. The chi-square test was used to assess the correlation between preoperative and postoperative parameters. The distributions of biochemical disease-free survival times were calculated according to the Kaplan-Meier curves and the logrank test was used to compare curves for groups. All p values less than 0.05 reflected statistically significant differences.

RESULTS

A total of 75 patients were entered in the trial. The prostate was treated in 1 (75) or 2 (14) HIFU sessions in a total of 89 procedures (1.2 sessions/patient). One patient with stage T1b, 1 patient with a serum PSA of 20.60 ng/ml and 1 patient on whom treatment was stopped during the procedure because of appearance with large microbubbles in the prostate were excluded. The median age, serum PSA level and prostatic volume of the 72 patients analysed were 72 yrs (range 45 to 79), 8.10 ng/ml (range 2.10 to 19.80) and 22.1 ml (range 8.5 to 52.8), respectively. The TNM stage was T1c in 40 patients, T2a in 18 patients and T2b in 14 patients. All patients had a histological diagnosis of prostatic adenocarcinoma according to the Gleason grading system. The Gleason score was 2 to 4 in 9 patients, 5 to 7 in 55 patients, 8 to 10 in 6 patients and unknown in 2 patients (Table 1).

The median time of HIFU treatment and hospitalization was 169 min (range 65 to 485 min) and 5.0 days (range 2 to 55), respectively. The gland size decreased from an initial volume of 24.2 ml to a final median volume of 14.0 ml ($p < 0.01$) in 45 patients. Totally, 49 out of 72 (68%) had negative follow-up biopsies at 6 months after HIFU. Biochemical disease-free survival rates were analyzed in 60 patients. Twelve patients were excluded from the analysis for unsatisfactory followup. The median follow-up period for all patients was 14.0 months (range 2 to 24). Biochemical disease-free survival rates in all patients at 1

Table 1. Characteristics in 72 patients with localized prostate cancer

Median age (range)	72 (45–79)
Median PSA (range)	8.10 ng/ml (2.10–19.80)
Prostate volume (range)	22.1 (8.5–52.8)
Pretreatment PSA (%):	
10 or less	44 (61)
10.1–20	28 (39)
Clinical stage (%):	
T1c	40 (56)
T2a	18 (25)
T2b	14 (19)
Gleason score (%):	
2–4	9 (13)
5–7	55 (76)
8–10	6 (8)
Unknown	2 (3)
Median mos followup (range)	14.0 (2–24)

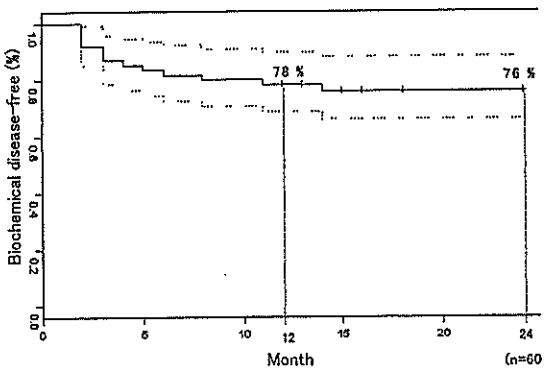

Fig. 3. Kaplan-Meier biochemical disease-free survival curves in all patients.

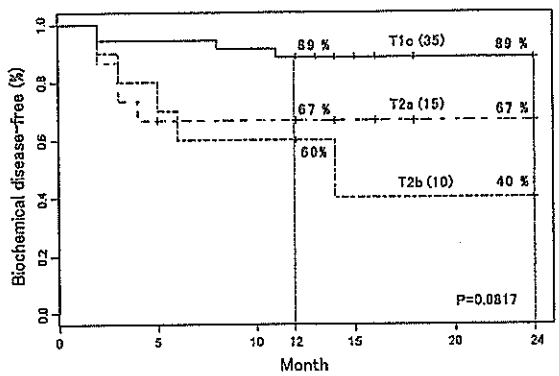

Fig. 4. Kaplan-Meier biochemical disease-free survival curves according to clinical stage.

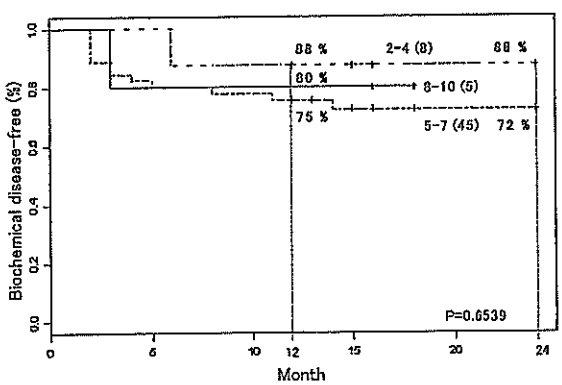

Fig. 5. Kaplan-Meier biochemical disease-free survival curves according to Gleason score.

and 2 years were 78% and 76%, respectively (Fig. 3). Biochemical disease-free survival rates in patients with stage T1c, T2a and T2b groups at 2 years were 89%, 67% and 40% ($p = 0.0817$, Fig. 4). Biochemical disease-free survival rates in patients with Gleason 2–4, 5–7 and 8–10 groups at 2 years were 88, 72% and 80% ($p = 0.6539$, Fig. 5). The biochemical disease-free survival rate in patients whose serum PSA less than 10 ng/ml and 10 - 20 ng/ml were 75% and 78% ($p = 0.6152$).

Prostatic volume was decreased from 24.2 ml to 14.0 ml at 6 months after HIFU ($p < 0.01$, Fig. 6). No statistically significant difference was noted in I-PSS, Q-max and FACT quality of life analysis (Fig. 7, 8 and 9).

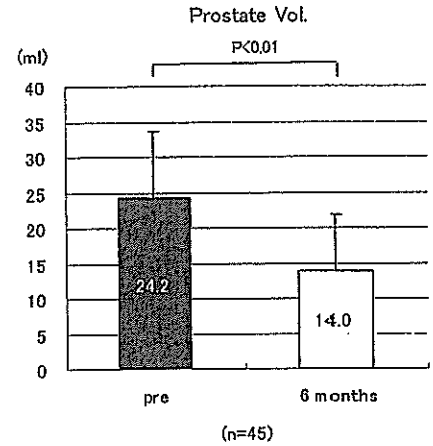

Fig. 6. Changes of prostatic volume.

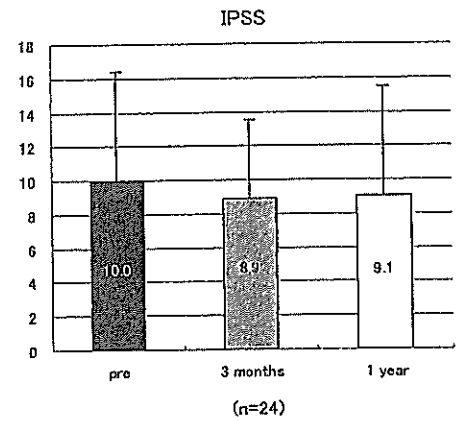

Fig. 7. Changes of International Prostatic Symptom Score.

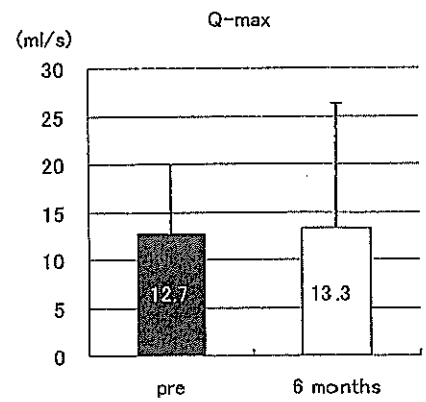

Fig. 8. Changes of maximum flow rate.

Thirteen out of 72 patients developed a urethral stricture, 6 and 4 patients developed epididymitis and prostatitis. Postoperative erectile dysfunction was noted in 12 out of 31 (39%) patients who were potent preoperatively. Nephrotic syndrome, transient urinary incontinence, transit stooly incontinence, balanoposthitis or retrograde ejaculation was observed in 1 patient each (Table 2).

For analysis of HIFU treatment using Sonablate 500^{TM}, ultrasound imaging for identifying prostate and quality levels were categorized more than good in patients with 92%. A transrectal probe was easily

Table 2. Complications

Complication	Grade 1	Grade 2	Grade 3	Grade 4	Total
Urethral stricture	0	0	13	0	13
Erectile dysfunction (31 potent patients)	0	0	12	0	12
Epididymitis	2	2	2	0	6
Prostatitis	2	0	2	0	4
Nephrotic syndrome	0	0	1	0	1
Balanoposthitis	1	0	0	0	1
Uninary incontinence (grade 1)	1	0	0	0	1
Stooly incontinence	1	0	0	0	1
Retograde ejaculation	1	0	0	0	1

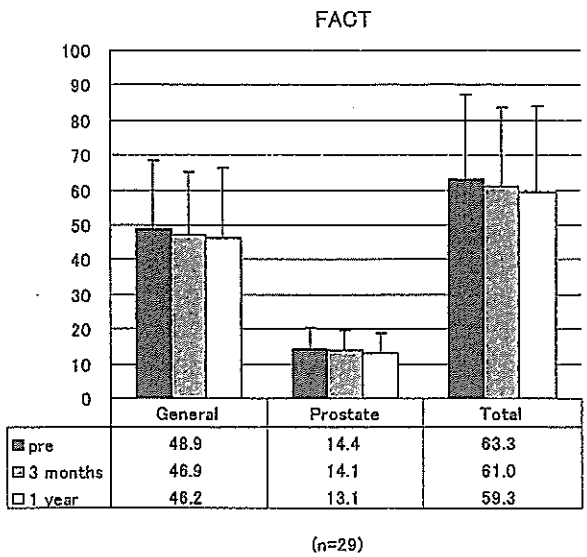

Fig. 9. Quality of life change by FACT general and prostate.

inserted into the rectum in 97% of the patients. Totally, 96% of the HIFU treatment was categorized as an easy procedure.

DISCUSSION

In 1995, Madersbacher et al. reported the effectiveness of HIFU in 10 cases of localized prostate cancer[8]. Histologically, HIFU-treated lesions of the prostate demonstrated a coagulation necrosis with sharp boundaries. In 1996, Gelet et al. reported preliminary experiences with HIFU using the Ablatherm device (EDAP-Technomed, Lyon, France) for treating localized prostate cancer[16]. Beerlage et al. reported the results of HIFU treatments in 111 patients with clinical stage T1-3N0M0 prostate cancer and a PSA level less than 25 ng/ml. The treatment for the first 49 patients was performed selectively (i.e. unilateral or bilateral treatment in one or two sessions depending on findings from TRUS and biopsies) and the whole prostate was treated in the remaining 62 patients. A complete response (defined as a PSA level < 4.0 ng/ml and a negative biopsy) was achieved in 60% of the whole prostate treated patients with and in 25% of selectively treated patients[17].

In 2001, Gelet et al. reported their long-term follow-up data in which a complete response was obtained in 66% of patients with no residual cancer (regardless of PSA levels) or no increases in PSA levels in three consecutive examinations with a PSA velocity < 0.75 ng/ml/year for patients with negative biopsies[18]. More recently, Chaussy and Thuroff summarized clinical outcomes by the ASTRO definition as 84.2% stability rate in the HIFU group and 80% rate in the combination with transurethral resection of the prostate (TURP) and HIFU group in 1 year[19]. In summarizing our clinical outcome using the ASTRO definition, the biochemically disease-free survival rate was 76% at 2 years follow-up. Patients with stage T1c, T2a and T2b showed resectively 89, 67% and 40% biochemical disease-free survival rates at 2 years follow-up ($p=0.0817$). The clinical outcome in our series of patients with preoperative PSA less than 20 ng/ml were comparable to the outcome of patients treated with radical prostatectomy[2,3].

In our series, postoperative urethral strictures at near verumontanum in the prostatic urethra occurred in 21% of the patients. Recently, TURP or bladder neck incision immediately before or after HIFU was found to reduce the treatment-related morbidity such as postoperative prolonged urinary retention, urinary catheterization time and urinary infection[20,21]. Neoadjuvant hormonal therapy also might be useful to reduce the volume of the prostate which can reduce the time of treatment and rate of morbidity. However, the upper limit of the gland volume is 50 ml even after reducing the size of the prostate with neoadjuvant androgen deprivation or TURP in our series. Generally, radicalism of prostate cancer and preservation of sexual function are always controversial because postoperative impotence depends on preservation of neuro-vascular bundles that sometimes includes tumor invasion. In our study, 39% of the patients exhibited erectile dysfunction after the HIFU therapy. One out of 12 patients who desired treatment for postoperative erectile dysfunction recovered with sildenafil citrate. We considered this rate to be lower than that compared to radical prostatectomy[2,3]. Further experience is required to confirm this important conclusion.

D'Amico et al. compared the outcome of a cohort

treated with 3D-CRT versus a matched cohort treated with brachytherapy plus external radiation therapy. The 5-year estimate of PSA failure-free survival rate after 3D-CRT alone was 45% and 67% when both radiation treatments were combined[22]. More recently, Kupelian et al. compared the biochemical disease-free survival rate after permanent seed brachytherapy, external beam radiation therapy (EBRT), combined seeds and EBRT, or radical prostatectomy for clinical stage T1-2 localized prostate cancer[23]. The 5-year biochemical disease-free survival rate for radical prostatectomy, EBRT <72 Gy, EBRT ≥ 72 Gy, permanent seed brachytherapy and combined seeds and EBRT were 81, 51, 81, 83% and 77%, respectively. Although not directly comparable, the results after treatment with HIFU appear to be similar to those after radiotherapy, even when both brachytherapy and EBRT are combined.

For many reasons, transrectal HIFU appears to be highly attractive as a minimally invasive treatment for localized prostate cancer. HIFU treatment requires no incision or puncture, with no bleeding, can be performed on an outpatient basis and is repeatable even when patients with local recurrence have already been treated with radiation therapy[24]. In addition, radiation therapy including brachytherapy and even surgery can be performed after HIFU.

Transrectal HIFU has considerable potential as a noninvasive treatment modality for patients with localized prostate cancer especially whose PSA less than 20 ng/ml.

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和文抄録

限局性前立腺癌に対する高密度焦点式超音波療法：多施設共同研究

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限局性前立腺癌に対する高密度焦点式超音波療法の多施設共同研究の成績について報告する．対象は，stageT1-2N0M0 の72例の限局性前立腺癌で，治療にはソナブレート500 (Focus Surgery, IN, USA) を用いた．効果判定には，American Society for Therapeutics Radiology and Oncology の効果判定基準を用いた．症例の年齢中央値は72歳，血清 PSA 中央値は 8.10 n/ml であった．また，術後観察期間中央値は14.0カ月間であった．治療効果は，全体では1年78%，2年76%が非再発生存であった．浸潤度別に2年目の生化学的非再発生存率を集計したところ，stage T1c が89%，stage T2a 67%，stage T2b は40% (p=0.0817) であった．悪性度別では，Gleason 2～4群は88%，Gleason 5～7群は72%，Gleason 8～10群は80% (p=0.6539) であった．術前の血清 PSA 値別2年非再発生存率は，PSA が10 ng/ml 以下群は75%，10～20 ng/ml 群は78% (p=0.6152) であった．術後6カ月目の前立腺生検では68%において癌細胞は認められなかった．前立腺体積は，術前 24.2 ml から術後6カ月目 14.0 ml と縮小していた (p<0.01)．IPSS，最大尿流量率，Functional Assessment of Cancer Therapy (FACT) を用いた生活の質項目は術前後に有意な変化は認められなかった．高密度焦点式超音波療法は，術前血清 PSA 値が 20 ng/ml 以下の限局性前立腺癌に対して低侵襲性でかつ有用な治療法と思われる．

(泌尿紀要 **51**：651-658，2005)

局所進行前立腺癌に対するホルモン療法と手術療法の併用療法

藤元博行

Radical prostatectomy with neoadjuvant hormone therapy for cT3 prostate cancer

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Urology Division, National Cancer Center Hospital

Abstract

The efficacy of neoadjuvant hormone therapy and radical prostatectomy for cT1−2 prostate cancer have been reported to be negative from some randomized prospective studies. On the other hand, radical prostatectomy alone for cT3 prostate cancer is understood as out of indication because of high rate of positive surgical margin and PSA failure. Several investigators have examined the role of neoadjuvant hormone therapy before radical prostatectomy for cT3 prostate cancer to improve outcome.

This document was reviewed the literature whether neoadjuvant hormone therapy is beneficial or not, for organ confined prostate cancer and for locally advanced prostate cancer, and presented our extended resection of prostate with neoadjuvant hormone therapy is improved the results in cT3 prostate cancer.

Key words: radical prostatectomy, neoadjuvant hormone therapy, surgical resection

はじめに

前立腺全摘(radical prostatectomy: RP)に先立ちある程度の期間，術前ホルモン療法(neoadjuvant hormone therapy: NHT)を実施することにより，downstaging(micrometastasisを消滅させることも含む)が起こることで治療成績の向上が期待されたが[1-4]，各種のランダム化試験ではその効果は否定的である[5-7]．

本稿ではまずNHTに関する各種の試験の結果を提示，考察する．最後にまだ経過観察期間が短くpreliminaryな結果ではあるが，NHTを施行した後，より確実な切除を目指した前立腺広汎全摘の成績を供覧して，局所進行前立腺癌に対する前立腺全摘除術の意味を考察したい．

1. 術前内分泌療法の治療成績

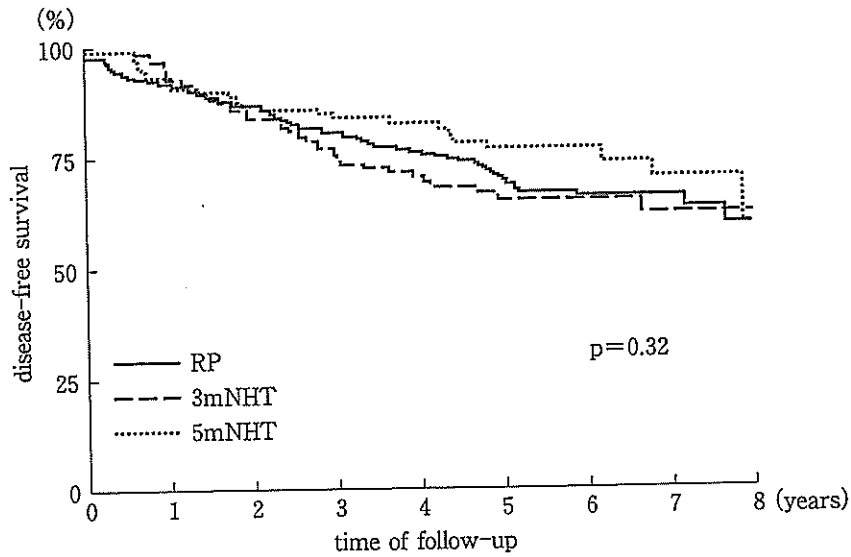
既に述べたようにNHTに関するランダム化試験の結果の長期成績ではその効果は否定的である．しかし，結果を解釈するときに注意が必要ではと考えている．一つはNHTの期間に関してである．多くのスタディでは3カ月程度のNHTが施行されている点である[5-7]．もちろんカナダでの3カ月と8カ月のNHTのランダム化試験[8]で8カ月のNHTでは切除断端陰性となりやすい(表1)が，切除断端が陰性となっても最終的にはPSA failureには関与しないのではと考えられている[9]ことも事実であるが，3カ月

国立がんセンター中央病院泌尿器科

0047-1852/05/¥50/頁/JCLS

表1 Statistically significant differences found : 3 versus 8 months' neoadjuvant hormone therapy (NHT)[8]

	3 months NHT	8 months NHT	p-value
presurgery PSA nadir level	35%<0.1ng/dl	73%<0.1ng/dl	<0.0001
TRUS prostate volume (mean)	40.5 cm^3 to 25.7 cm^3 (37%)	40.5 cm^3 to 22.8 cm^3 (48%)	0.0001
positive margins after surgery	23%	12%	0.01



	Hazard ratio	95% CI
RP alone	1	
3mNHT	1.01	0.70-1.45
5mNHT	0.60	0.38-0.94

図1 Kaplan-Meier curves for disease-free survival until PSA failure and Hazard ratios[9]

3m: 3 months, 5m: 5 months, NHT: neoadjuvant hormone therapy, RP: radical prostatectomy

程度のNHTでは効果が期待できないとの報告もある[9]. この報告によるとRP単独に対して3カ月のNHTはhazard ratioが1.01, 5カ月のNHTでは0.60となっており(図1), 適切なNHT期間に関するエビデンスは乏しいと思われる.

またそもそもスタディの対象としている病態についても注意が必要ではと考えている. もともと前立腺癌の術前病態は過少評価される傾向があることより, 本来のRPの適応と考えられるT1-2主体のスタディと逆に, T3, T4といった本来monotherapyでは限界があるとされる局所進行前立腺癌を対象にし, その生存率の向上を狙ったスタディかという点である. 多くのランダム化[5-7]あるいはphase IIスタディ[10-12]では前者を対象としている. つまりスタディコンセプトとしてはT2癌の20-30%がpT3であり, NHTを施行することでdownstagingが起こり, pT3前立腺癌がpT2, つまり本来の前立腺全摘の適応となるのではということを期待したスタディである. しかし結果的にNHTによるdownstagingは期待できず, またNHTにより切除断端陽性(positive surgical margin: PSM)が回避

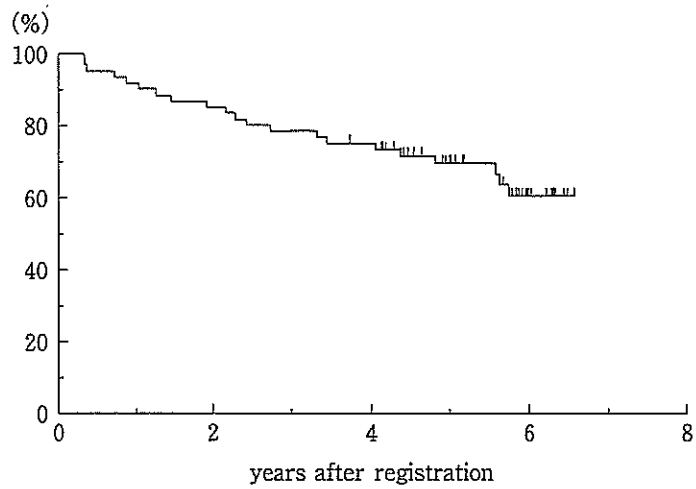


図2 Progression-free survival in SWOG 9109 study[17]

されてもNHTによるartifactであり，PSA failureを回避することはできないとの結論[13-15]となっていると解釈される．実際NHTによりpT0となっていても2割程度に再発を来すことがあり，このデータはRP単独と同様ではと失望させられるという報告[16]もある．更に詳細は不明な文献もあるが，このような疾患を対象として実施された手術は神経温存前立腺全摘が大半であると想定される点である．downstagingが起こらなければ，pT3前立腺癌に対して神経温存手術を行うことはPSMを来す危険性があることは当然である．

以上の結果は，cT1-2前立腺癌に対してdownstagingを狙ってNHTを施行してもPSMをなくすことで治療成績を向上させるという目的は無効であるという解釈となる．

2. 局所進行前立腺癌に対する内分泌療法併用前立腺全摘の成績

本来手術の適応と考えられるcT1-2前立腺癌に対して，治療成績の向上を狙ったNHTの試みはnegativeな結果となったわけであるが，局所進行前立腺癌に対する治療成績を考察するうえで注意を有するのはNHTの後に施行される前立腺癌全摘においてどのような立場で手術がなされたかという点である．cT1-2においては当然，神経温存前立腺全摘，これが標準の手術療法というのがコンセンサスであり，cT3においても，NHTによりdownstagingを来すことにより，このような手術でも対応可能としてスタディがなされたのか，cT3では神経温存を目的とせず，より切除断端を確保すべくwide resectionがなされたか否かという点である．

この点でcT3を対象としたNHTのphase IIスタディとしてSouthwest Oncology Group (SWOG) Study 9109[17]の結果とWalshらが確立した前立腺全摘を施行したGomellaら[18]の結果の比較は興味深い．図2にSWOG studyの結果と図3にGomellaの結果を示した．Gomellaのスタディは症例数が少なく，背景も違うことから単純な比較はもちろんできないのであるが，結果の違いはあまりに大きい．SWOG studyではPSMの率が明らかに他のスタディと比較して低く，その理由として切除断端を広くとる努力がなされたことによるのではと考察している．

このようにNHTを施行した後に実施する前立腺全摘の方法により治療成績が異なる可能性は十分に考えられる．一方ではNHTを施行しなくても神経温存を行わない前立腺全摘によって同様の結果が得られるのではとの仮定もあるが，この点を比較した試験はないように思われる．

3. 術前内分泌療法を併用した広汎前立腺全摘の治療成績

確実な切除断端を追求することは，治療成績

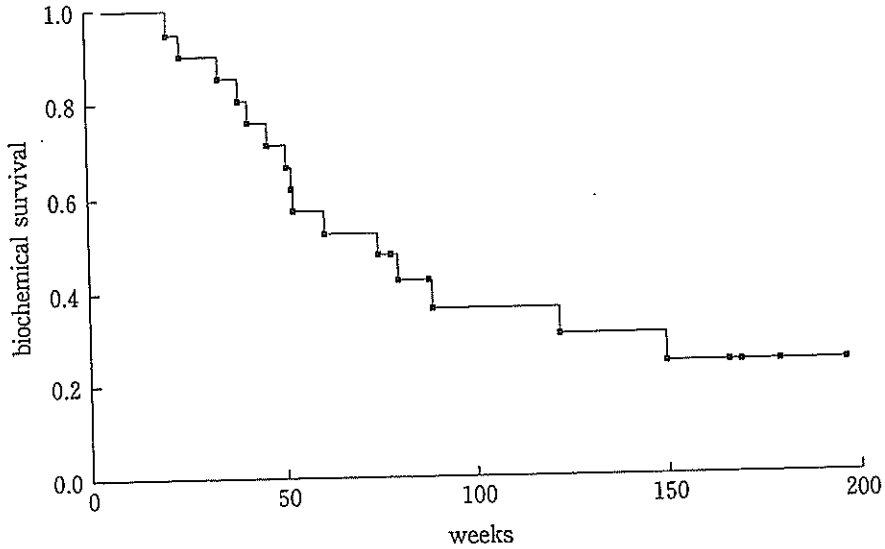


図3 cT3 prostate cancer: freedom from biochemical relapse[18]

の向上につながる可能性があるはずである．もちろん細胞学的な転移があり，局所切除を追求してもその治療成績の向上につながらない病態が存在することは事実であるが，逆に局所施行癌であっても切除が可能な病態もあることも事実であり，著者らは局所の切除をより完全に行うことで局所進行前立腺癌に対して根治の可能性を追求してきた．このアプローチに関するスタディコンセプトを以下に述べる．

これまでのNHTのスタディの結果からdown-stagingはあまり期待せず，したがってNHTの効果を期待して縮小手術を行うのは危険である．NHTを施行することにより前立腺体積が減少することは明らかである．また確実な切除断端を確保することは治療成績の向上につながることも明らかである．したがってNHTはdown-sizingを目的に併用することで相対的に広汎な切除断端を確保することが可能となり，治療成績が期待できるのではと考えた．そもそも日本人の骨盤は狭く，大きな前立腺を摘出する場合には広く切除断端を確保することが困難である．また前立腺全摘において尖部の処理は断端の確保のみではなく，機能温存，出血量のコントロールなどに重要であることはいうまでもない．近年ではPSAにより発見される前立腺癌が増えており，このような病態では前立腺尖部腹側に病巣が多く存在することが認識されている[19]．前立腺尖部と恥骨との間が拡大することにより少しでも距離が確保されることは切除に際して有利に働くはずである．また前立腺尖部が縮小することで相対的に尿道括約筋が長く温存できる可能性が高くなり，術後の尿禁制に対しても有利に作用すると考えられる．

NHTを施行することにより前立腺周囲に線維化が起こることによる手術の困難性が指摘されているが[13,14]，局所進行前立腺癌に対しては神経を温存することはその治療的意義から疑問があり，原則実施していないこと，精嚢は周囲から広汎に切除すること，更には手術に対する慣れもあり，著者らは特に困難を感じることはない．

またこれは我が国の患者の特徴の一つではないかと思われるが，性機能温存に対してそれほどのこだわりがなく，むしろ手術により癌根治を望む症例が多いことも本スタディを可能とした要因である．

以上のコンセプトに基づき，より確実な切除断端を確保する手術法'広汎前立腺全摘：extended radical prostatectomy'[20]を開発し，cT3前立腺癌に対するNHT併用手術療法の治療を行ってきた．

a. 対象と方法

2000年からは術前3カ月以上，1年以内，推奨6カ月の術前ホルモン療法を施行した後，前

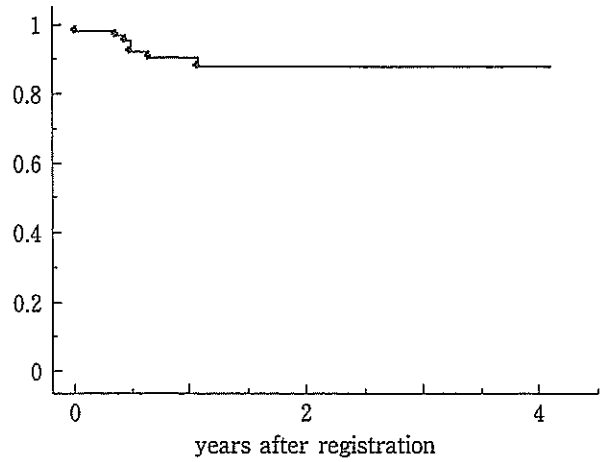


図4 Biochemical-free survival by extended radical prostatectomy in 70 cT3 prostate cancer

立腺全摘を施行し，病理結果のいかんにかかわらず術後は無治療経過観察を行うphase IIスタディを施行している．今回，cT3N0M0前立腺癌に対して上記のプロトコールで治療を行った広汎前立腺全摘症例70例の治療成績を検討した．後述するように本術式の適応拡大を狙った局所の相当な進行癌(TxN0M0)に対してトライアル的に実施(phase II後期)した症例は除いている．広汎前立腺全摘に関する詳細は文献[20]に記載しているが，その概略を述べると，直腸固有筋膜を切開し，直腸筋層を露出し，剥離を進め，腱中心に至ることで前立腺尖部後面の把握を確実にする．神経を含む血管束を可及的末梢で完全切除するとともに，前立腺尖部を直腸の剥離を参考にしながら，前立腺尖部後面と腱中心との間を安全，確実に切断する．中枢に向かい逆行性処理を行い，腹膜翻転部を確認して精嚢基部を露出することなく，また膀胱頸部を大きく切開し，膀胱筋層も含めて前立腺を摘出する手術手技である．

平均年齢は64歳，治療前PSA値は2.4-124 ng/dl，Gleason scoreは6-9であり，平均観察期間は581日(112-1,500日，中央値455日)である．

b. 結果と考察

摘出標本における70例のpT分類はpT0: 1例，pT2a: 2例，pT2b: 25例(pT2: 27例(38.6%))，pT3a: 20例，pT3b: 6例(pT3: 26例(37.1%))，pT4: 16例(22.9%)である．当然，術前診断の正当性が問題になるわけであるが，例えばcT3前立腺癌に対するNHTとして術後pT分類が記載されているEuropean Study Group[21]の結果と比較してみると，NHTの期間に差があり単純な比較はできないのであるが，少なくともpT3以上の病期が50%近くを占めており，特に著者らの臨床診断がoverstagingであるということではないと思われる．著者らの症例の40%近くがpT2と診断された症例が多いことはNHTの期間にも起因しているとも考えられるのであるが，一般的にcT3に対しても15-25%のoverestimationがあるとされており，NHTの効果とステージングエラーの両方を含む症例数としては理解可能な数字ではないかと考える．

図4に全体の成績を示す，PSA failureを0.2 ng/dl以上として検討した結果，88.4%がNEDの状態であり，12%にPSA failureを認めた．摘出標本における病期別の治療成績を図5に示す．興味深いことにpT2a-pT3aでは非常に良好な治療成績であり，局所限局癌と遜色がない．pT2a-pT3a全体で49例中，1例にのみPSA failureを認めている．pT3b 6例中2例，pT4 14例中4例にPSA failureを認め，この群では2例にリンパ節転移を認めている．リンパ節転移陽性例は全例400日以内にPSA failureとなったが，このことは当然のことと考えられた．PSMはNHTを施行しないRP時の大きな予後規定因子

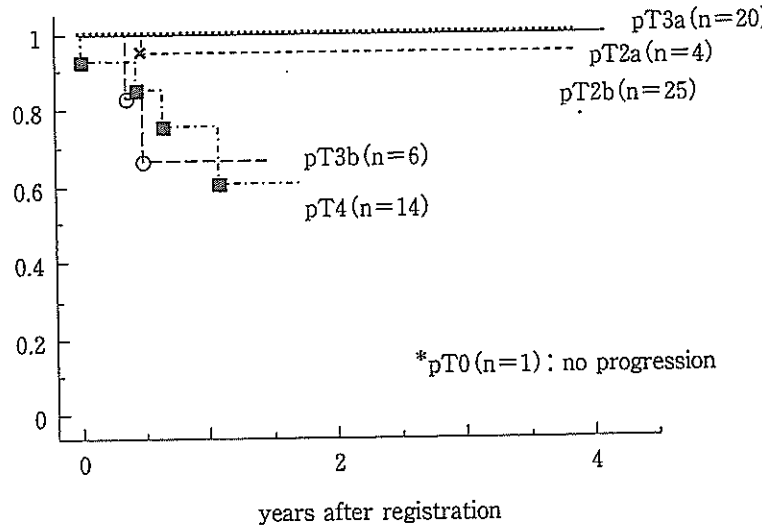


図5 Biochemical-free survival according to the pT stage by extended radical prostatectomy in cT3 prostate cancer

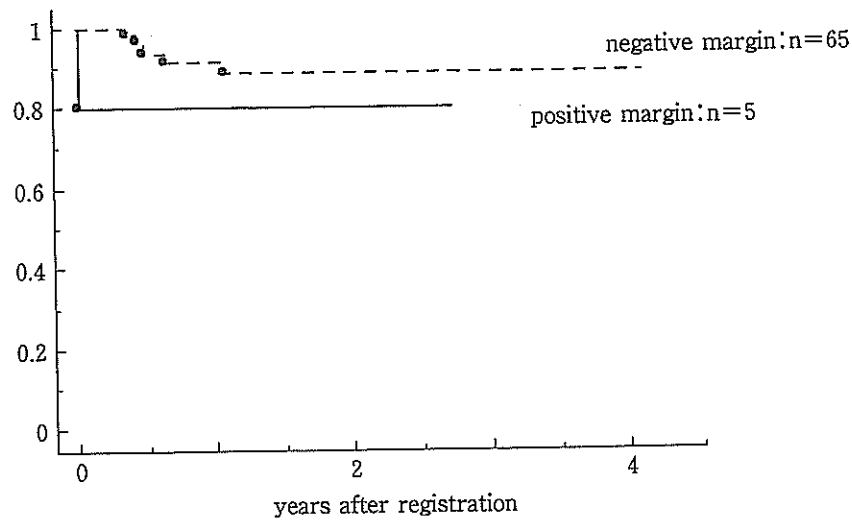


図6 Biochemical-free survival according to the surgical margin by extended radical prostatectomy in cT3 prostate cancer

となるが，NHT下のRPではその判定がartifactなども加わり明確な予後規定因子とならないとの意見[13-15]もあるが，今回の症例群で明らかなPSMは5例に認められ，そのうち1例がPSA failureであった．逆に65例はnegative surgical marginと判断されたがそのうち6例にPSA failureが認められた．図6にその成績を示す．ある程度の傾向は認められるが，negative surgical marginでも10%程度にPSA failureが認められたことになり，pT0となった場合でも再発することが十分あり得る[16]前立腺癌においては病理学的にnegative surgical marginと判断されても決して再発の危険が少ないことを意味するものではないことがやはり伺える．

いずれにしてもこの結果はcT3を対象にしたRPの成績としてはまだ観察期間が短いという問題はあるが文献的にみてもbest resultになるのではないかと考えられる．

我が国の前立腺癌症例においてはPartin nomogram[22]と比較してもnode positiveが低いという印象があり，局所の完全切除を追求することには治療的意義があると考えられる．ただ

し多くの文献でも指摘されているように，cT2前立腺癌にも約3割のunderestimationがあるように，cT3前立腺癌に対しても15-25%のoverestimationがあり得るし，cT3前立腺癌にもunderestimationがあり得る．今回の対象症例も画像などから被膜浸潤ぎりぎり陽性と判定された'早期'のT3症例と，生検などから相当の腫瘍量が想定され，実際，術後pT4と診断された'相当な'T3症例が混在しており，cT3前立腺癌にはcT2前立腺癌以上にその病態はheterogenousな状態と考えられる．cT3前立腺癌で真に局所切除の意味がある症例群の解析が今後の課題と考えており，現在施行している局所の'相当な'進行癌(cTxN0M0癌)に対する広汎前立腺全摘のトライアルスタディの結果を待つ必要がある状況である．

また当初，NHTを併用する意義としてdown-sizingによる相対的な切除断端の確実な確保を目的としていたが，元々前立腺の体積の小さな症例では不要ではという想定もあり，広汎全摘におけるNHTの意義を確認する研究が必要とも考えている．

おわりに

cT3前立腺癌に対するNHTを併用したRPには限界があり，NHTの意義は見いだせないとの意見が多いが，局所をより完全に切除することにより，治療成績の向上が期待できないかとの問題意識から広汎前立腺全摘を開発した．この手術法をもってcT3前立腺癌に対して治療を行ったところ，cT3症例でもpT3aかそれ以下ではorgan confined diseaseと治療成績が全く異ならないことを確認した．我が国の前立腺癌患者では性機能障害よりも手術により根治を望むことが多く，また我が国の前立腺癌のリンパ節転移頻度は低いと想定され，このような環境下では欧米と異なり，cT3という理由で'手術療法ではもはや根治不可'と断定することはできないことを示した．

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