not correlated with the changes in serum dehydroe-piandrosterone, dehydroepiandrosterone sulfate, or androstendione, although some non-responders did not show significant suppression of adrenal androgens after dexamethasone therapy (data not shown). The change of serum IL-6 during dexamethasone therapy was evaluated in 16 patients. As shown in Figure 1, 5 of 8 responders to dexamethasone therapy demonstrated 80% or more decrease in serum IL-6 at 1 month from the start of dexamethasone. On the contrary, none of 8 non-responders showed remarkable IL-6 suppression (Fig. 2). There was an association between the response of dexamethasone therapy and 80% or more suppression of serum IL-6 ($P < 0.05$, chi-square test).

DISCUSSION

In the present study, the favorable effect of low-dose dexamethasone was demonstrated in a substantial number of patients who showed PSA failure after initial endocrine therapy. Some of the previous studies also reported the high rate of PSA response to gluco-corticoid therapy in patients with progressive prostate cancer after androgen ablation [4–6]. Thus, it may be worthwhile to administer dexamethasone after confirming the antiandrogen withdrawal syndrome, since low-dose dexamethasone therapy does not have severe adverse effects.

The mechanism of dexamethasone therapy for hormone-refractory prostate cancer has been believed to be suppression of adrenal androgens. However, in the majority of patients in the present series, serum levels of adrenal androgens were suppressed by
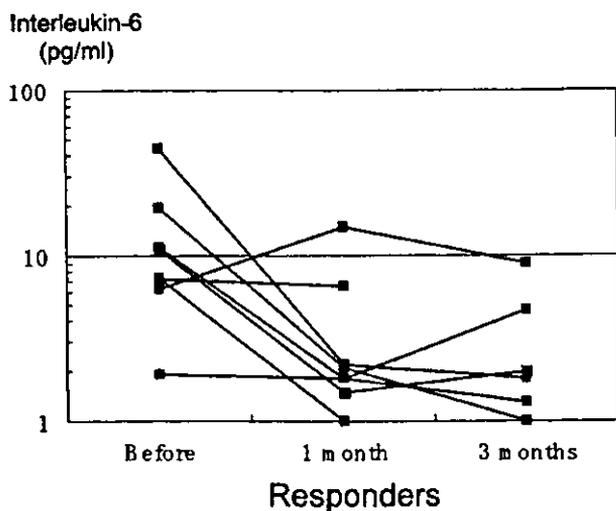
Interleukin-6
(pg/ml)

Fig. 1. Change of serum interleukin-6 (IL-6) following dexamethasone therapy. Each dot represents each patient. Responders to dexamethasone therapy (n = 8): patients who showed 50% or more decline of serum PSA and/or improvement of pain.
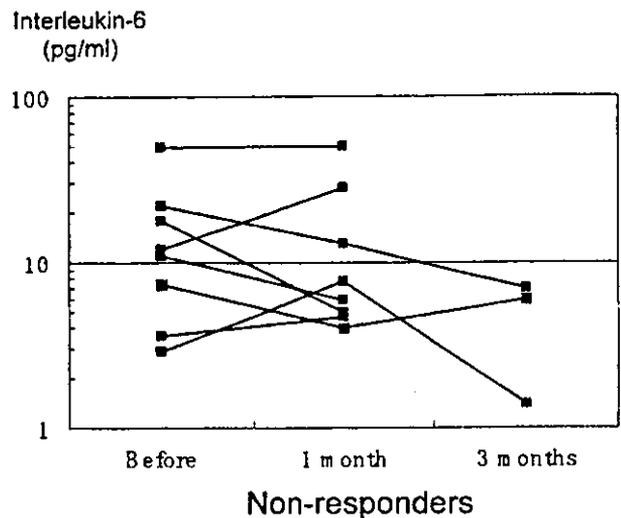
Interleukin-6
(pg/ml)

Fig. 2. Change of serum IL-6 following dexamethasone therapy. Each dot represents each patient. Non-responders to dexamethasone therapy (n = 8).

dexamethasone therapy irrespective of the response of dexamethasone therapy. In some of non-responders to dexamethasone therapy, no marked suppression of adrenal androgens was observed, probably due to low compliance with dexamethasone administration, since serum cortisol was not decreased very much.

The present study suggests another possible mechanism of dexamethasone action, that of significant suppression of IL-6. The direct effect of dexamethasone on prostate cancer cells has been suggested through NF-kappaB–IL-6 pathway [7]. However, circulating IL-6 is thought to be derived from many different cell types including monocytes, fibroblasts, endothelial cells, and possibly some of prostate cells [8]. IL-6 is known to be suppressed by glucocorticoids [9] and to stimulate the growth of the prostate cancer cell lines through its receptors in an androgen-independent manner [10–12]. In addition, recent reports have shown that IL-6 can activate the androgen receptor through a signal transducer and activator of transcription 3 (STAT3)-dependent pathway [13–15]. Circulating IL-6 levels are high in hormone-refractory prostate cancer patients [16], and serum IL-6 may be a good prognostic factor after androgen ablation therapy in prostate cancer patients [17]; the present study shows that remarkable suppression of serum IL-6 is closely related to the response to dexamethasone therapy. Since serum level of IL-6 was not related to serum PSA at the start of dexamethasone therapy in the present study, and both IL-6 producing and non-producing prostatic cancer cells have been reported in the literature [10], it seems unlikely that the decline of serum IL-6 simply resulted from reduction of IL-6

producing cancer cells. Therefore, the decrease in circulating IL-6 by dexamethasone administration may reflect inhibition of ligand-independent activation of the androgen receptor, resulting in inhibition of expression of androgen responsive genes.

The androgen receptor plays a key role in androgen-dependent proliferation of prostate cancer cells. Although the content of androgen receptor has been shown to be a prognostic indicator in prostate cancer patients treated by endocrine therapy, androgen-independent tumors can express the androgen receptor [18], suggesting that post-receptor pathways of cell proliferation are preserved in a number of prostate cancer cells. Therefore, it is likely that expression of other androgen-responsive genes which control androgen-dependent proliferation of cancer cells could be similarly inhibited by dexamethasone.

CONCLUSIONS

The favorable effect of low-dose dexamethasone was demonstrated in prostate cancer patients who showed PSA failure after initial endocrine therapy. Significant suppression of IL-6 may represent one of the mechanisms for the effect of dexamethasone therapy in prostate cancer patients showing biochemical failure. Since the present study was based on a small number of patients and the observation seemed preliminary, further investigations would be required to make final conclusions.

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Clinical Trial Note

Randomized Controlled Trial to Evaluate Radiotherapy ± Endocrine Therapy Versus Endocrine Therapy Alone for PSA Failure after Radical Prostatectomy: Japan Clinical Oncology Group Study JCOG 0401

Akira Yokomizo[1], Hiroshi Kawamoto[2], Keiji Nihei[3], Naoki Ishizuka[2], Yoshiyuki Kakehi[4], Ken-ichi Tobisu[5] and Seiji Naito[1]

[1]Department of Urology, Graduate School of Medical Science, Kyushu University, Fukuoka, [2]Cancer Information and Epidemiology Division, National Cancer Center Research Institute, Tokyo, [3]Radiation Oncology Division, National Cancer Center Hospital East, Kashiwa, Chiba, [4]Department of Urology, Faculty of Medicine, Kagawa University, Kita-gun, Kagawa and [5]Shizuoka Cancer Center Hospital, Sunto-gun, Shizuoka, Japan

A randomized controlled trial has started in Japan to evaluate radiotherapy and endocrine therapy for prostate-specific antigen (PSA) failure after radical prostatectomy. Patients who have PSA failure after radical prostatectomy for localized prostate cancer (T1–2N0M0) are randomized into treatment groups of either radiotherapy ± endocrine therapy or endocrine therapy alone. The Urologic Oncology Study Group (UOSG) in the Japan Clinical Oncology Group (JCOG) composed of 36 specialized institutions will recruit 200 patients. The primary end-point is time to treatment failure (TTF) of bicalutamide, and secondary end-points are TTF of protocol treatment, progression-free survival, overall survival, adverse events and quality of life (QOL). The Clinical Trial Review Committee of the JCOG approved the protocol on April 13, 2004, and the study was activated on May 17, 2004.

PROTOCOL DIGEST OF THE JCOG 0401

TRIAL BACKGROUNDS

In spite of improvements in both the detection of early prostate cancer and surgical techniques, ~35% of men develop prostate-specific antigen (PSA) failure after radical prostatectomy (1). Most of the recurrences after radical prostatectomy are detected only by a rise in the PSA level (2). Those who have local recurrence may benefit from radiation therapy, whereas those who have metastatic disease may benefit from systemic treatment, the most common of which is androgen deprivation (2). As computed tomography (CT) scans or bone scans usually cannot detect the recurrent sites, a standard has not yet been established for the treatment of PSA failure after prostatectomy.

For reprints and all correspondence: Seiji Naito, Department of Urology, Graduate School of Medical Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: naito@uro.med.kyushu-u.ac.jp

PURPOSE

The purpose of the trial was to evaluate radiotherapy ± endocrine therapy in comparison with endocrine therapy alone for PSA failure after radical prostatectomy.

STUDY SETTING

The study was a multi-institutional (36 specialized centers), randomized controlled trial.

RESOURCES

END-POINTS

In general, overall survival (OS) is supposed to be the best primary end-point to compare the clinical advantage in

randomized trials. However, the 10 year overall survival rate is expected to be >80% in this study, therefore OS will not be a good candidate for the primary end-point. The clinical progression-free survival is also not adequate as the primary end-point for the same reason. Regarding 'PSA failure', it may be a potential candidate for the primary end-point, but PSA failure will occur at least three times more frequently in the experimental arm, which causes confusion in evaluation. Therefore, the adequate primary end-point would be time to treatment failure (TTF) of luteinizing hormone-releasing hormone (LH-RH) analog as a hormone-refractory state of prostate cancer. As the TTF of bicalutamide can be evaluated more quickly than that of LH-RH analog and thus should be its good surrogate end-point, the TTF of bicalutamide is selected as a primary end-point in this study. In summary, the primary end-point is the TTF of bicalutamide, and secondary end-points are TTF of protocol treatment, clinical progression-free survival, OS, adverse events and patient-reported quality of life (QOL).

ELIGIBILITY CRITERIA

Tumors are staged according to the General Rule for Clinical and Pathological Studies on Prostate Cancer (Japanese Urological Association, The Japanese Society of Pathology), which is the 1997 revision of the TNM Classification of Malignant Tumours by the International Union Against Cancer (UICC) (3).

INCLUSION CRITERIA

(i) A diagnosis of localized prostate cancer (clinical stage T1–2N0M0) which was treated by radical prostatectomy; (ii) pathological stage: pT0/2/3 and pN0/x; (iii) the serum level of PSA once it has reached <0.1 ng/ml after radical prostatectomy and then increased to ≥0.4 ng/ml; (iv) a serum level of PSA ≤1.0 ng/ml at study entry; (v) no clinical recurrence based on abdominal and pelvic CT, and a bone scan; (vi) no history of chemotherapy, radiation therapy or endocrine therapy for any cancer; (vii) age ≥20 and ≤79 years; (viii) an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; (ix) no blood transfusion within 28 days of entry; (x) sufficient organ function within 28 days of entry; and (xi) written informed consent.

EXCLUSION CRITERIA

(i) Synchronous or metachronous (within 5 years) malignancy other than carcinoma *in situ*; (ii) mental disease or mental symptoms which would affect the participant's decision to participate; (iii) continuous medication of steroids (exclude external use of steroids for skin); (iv) ischemic heart disease or arrhythmia which needs medical treatment; (v) poorly controlled hypertension; (vi) poorly controlled diabetes mellitus; (vii) history of cerebral infarction or myocardial infarction within 6 months; (viii) liver cirrhosis; and

(ix) interstitial pneumonia which requires ventilation assistance, oxygen inhalation, steroids or diuretic medicine.

RANDOMIZATION

Using telephone or fax contact with the JCOG Data Center after confirmation of the above criteria, patients are randomized by the minimization method of balancing the groups according to the Gleason score of the radical prostatectomy specimen, period from operation to PSA failure, and institution.

TREATMENT METHODS

Endocrine therapy alone group (standard arm). The protocol treatment includes the bicalutamide medication (80 mg/day). After TTF of bicalutamide, it is followed by LH-RH analog (leuprorelin acetate 3.75 mg/4 weeks or 11.25 mg/12 weeks, goserelin acetate 3.6 mg/4 weeks or 10.8 mg/12 weeks).

Radiotherapy ± endocrine therapy group (experimental arm). The total dose of 64.8 Gy/36 Fr (50 days) external beam irradiation is delivered to the prostatic bed. If the patient has no treatment failure, no additional therapy will be given. In case of treatment failure of radiation therapy, bicalutamide medication will be started in the same way as in the standard arm. After the treatment failure of bicalutamide, a LH-RH analog is given to the patients as in the case of endocrine therapy alone.

DEFINITION OF TREATMENT FAILURE

(i) PSA increase beyond 0.4 ng/ml if previous value is <0.4 ng/ml
(ii) Any PSA increase if previous value is ≤0.4 ng/ml
(iii) Clinical progression or clinical recurrence
(iv) Adverse event
(v) Patient refusal to continue treatment
(vi) Any cause of death
(vii) Poor compliance (less than two-thirds of planned dose) of oral bicalutamide at two consecutive visits (only for bicalutamide treatment failure)

FOLLOW-UP

All patients are followed-up by their urologist at least every 3 months for more than 5 years. Blood tests including PSA and urinalysis are performed during the follow-up interval. Abdominal and pelvic CT, chest X-ray and bone scan are carried out every 12 months. The symptoms and adverse events are surveyed at each visit.

STUDY DESIGN AND STATISTICAL METHODS

This trial is designed to evaluate the superiority of radiotherapy ± endocrine therapy to endocrine therapy alone in terms of the TTF. Almost half of the patients can be cured by radiation therapy alone (4–6), therefore, these patients are

expected to have a greatly prolonged TTF after radiation (radiation responder). In contrast, the other half of the patients irradiated are expected to have a treatment failure of radiation therapy (non-responder) and they will have a TTF not significantly shorter than that of those on bicalutamide therapy. In the standard arm, there have been no published data concerning the TTF of bicalutamide for PSA failure after radical prostatectomy. Therefore, we assumed the TTF of bicalutamide therapy in this study to be 4–5 years, based on the report in which the median TTF of bicalutamide therapy for localized prostate cancer was 63.5 months (7). The median TTF in the experimental arm can be calculated on the assumption that the TTF in a radiation responder (50% of the experimental arm) is prolonged three times more than in the non-radiation responders (50% of the experimental arm). Therefore, the median TTF in the experimental arm will be 6.6 years (4.0 years in non-responders and 12.0 years in responders) and 8.3 years (5.0 years in non-responders and 15.0 years in responders). We calculated sample sizes based on Shoenfeld and Richter's methods (8) with 5 year follow-up after 4 years of accrual. If the TTF in the standard arm is 4.0 years, the detectable difference in TTF and sample size per arm will be 2.6 years and 83 cases, respectively. If TTF in the standard arm is 5.0 years, the detectable difference in TTF and sample size per arm will be 3.3 years and 93 cases, respectively. This will provide an 80% power to detect the difference between the assumed TTF in the experimental arm and the TTF in the standard arm (non-responder in the experimental arm compatible) at a 5% one-sided alpha level. Based on these data, the planned sample size is 100 cases in one arm.

QOL

All the patients are enrolled prospectively in a QOL survey using a validated assessment tool and are evaluated before the treatment and 1-year after the treatment. The health-related QOL is assessed using the Japanese version of the RAND Health-Item Short Form 36 (SF-36) version 2.0 (9), and cause-specific QOL is analyzed by the UCLA Prostate Cancer Index which was established by Litwin et al. (10). The Japanese version of SF-36 and that of UCLA PCI were assessed as described previously (11–13).

INTERIM ANALYSIS AND MONITORING

An interim analysis is planned to be performed once, taking into account multiplicity using the Lan and DeMets approach. The Data and Safety Monitoring Committee (DSMC) of the JCOG independently reviews the interim analysis report, and an early termination of the trial may be considered at that stage. In-house interim monitoring is performed by the Data Center to ensure data submission, patient eligibility, protocol compliance, safety and on-schedule study progress. The monitoring reports are submitted to and reviewed by the UOSG and the DSMC every 6 months.

PARTICIPATING INSTITUTIONS (FROM NORTH TO SOUTH)

Hokkaido University, Sapporo Medical University, Tohoku University, Miyagi Cancer Center, Akita University, Tsukuba University, Tochigi Cancer Center, Gunma University, Chiba Cancer Center, Chiba University, National Cancer Center Hospital, Tokyo Women's Medical School, Keio University, The Jikei University, Nippon Medical School, Kitasato University, Niigata Cancer Center Hospital, Niigata University, Yamanashi University, Shinshu University, Hamamatsu Medical School, Shizuoka Cancer Center, Nagoya University, Mie University, Kyoto University, Osaka Medical Center for Cancer and Cardiovascular Diseases, Kobe University, Nara Medical University, Shimane University, Kurashiki Central Hospital, Okayama University, Kagawa University, National Shikoku Cancer Center, Kyushu University, Kurume University and Kagoshima University.

A. Yokomizo, Study Coordinator; H. Kawamoto, Protocol Coordinator; K. Nihei, Radiation Oncology Study Coordinator; N. Ishizuka, Study Statistician; Y. Kakehi, QOL Coordinator; K. Tobisu, Chair of Urologic Oncology Study Group; S. Naito, Study Chair.

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Effectiveness of Adjuvant Intermittent Endocrine Therapy Following Neoadjuvant EndocrineTherapy and External Beam RadiationTherapy in Men With Locally Advanced Prostate Cancer

Hidetoshi Yamanaka,[1] Kazuto Ito,[2]* Seiji Naito,[3] Taiji Tsukamoto,[4]
Michiyuki Usami,[5] Hiroyuki Fujimoto,[6] Naoki Matsuoka,[6] Iwao Fukui,[7]
Masaoki Harada,[8] Yasuo Ohashi,[9] Toshihiko Kotake,[10] and Tadao Kakizoe[6]

[1]*Institute for Preventive Medicine, Kurosawa Hospital, Takasaki, Japan*
[2]*Department of Urology, Gunma University Graduate School of Medicine, Maebashi, Japan*
[3]*Department of Urology, Graduate School of Medicine, Kyusyu University, Fukuoka, Japan*
[4]*Department of Urology, Sapporo Medical University School of Medicine, Sapporo, Japan*
[5]*Department of Urology, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan*
[6]*Urology Division, National Cancer Center Hospital, Tokyo, Japan*
[7]*Department of Urology, Cancer Institute Hospital, Tokyo, Japan*
[8]*Kanagawa Cancer Center Research Institute, Yokohama, Japan*
[9]*Department of Hygiene and Preventive Medicine, School of Health Sciences and Nursing,
University of Tokyo, Tokyo, Japan*
[10]*Kotake Clinic, Osaka, Japan*

PURPOSE. To clarify the optimal duration and methods for adjuvant endocrine therapy after external beam radiation therapy (EBRT) in patients with locally advanced prostate cancer.
MATERIALS AND METHODS. Between 2001 and 2003, 215 patients with locally advanced prostate cancer were enrolled in the study. Patients were registered as primary candidates of the study and were treated with 6 months of LHRH agonist, with short-term of antiandrogen treatment for flare-up prevention. Patients with PSA levels below 10 ng/ml after the 6-month endocrine treatment were randomly divided into two arms. Then, a total dose of 72 Gy was given to the prostate. After 14 months of the protocol treatment, patients were treated with continuous androgen ablation (arm 1) or intermittent androgen ablation (arm 2).
RESULTS. A total of 188 cases (87%) remained in the protocol. The median PSA level at entry was 25.3 ng/ml. The Gleason score was 2–6 in 32 cases (16%), 7 in 94 cases (48%), and 8–10 in 68 cases (35%). The median PSA level showed a remarkable decrease to 1.1, 0.2, and 0.1 ng/ml, after 6, 8, and 14 months of the protocol treatment, respectively. Of the 157 cases treated with EBRT, 153 cases (97.5%) had no biochemical failure in the mean follow-up of 17.3 months.

CONCLUSIONS. The present study may reveal the possibilities of intermittent endocrine therapy after EBRT. However, the follow-up interval is short and little can be said about the results observed so far, exception of acute tolerance and patient acceptance of the protocol. *Prostate 63: 56–64, 2005.* © 2004 Wiley-Liss, Inc.

KEY WORDS: prostate cancer; intermittent hormonal therapy; external beam radiation therapy

INTRODUCTION

Treatment of prostate cancer has been one of the most important issues for elderly males, especially in Western countries. In Japan, prostate cancer is the eighth leading life-threatening cancer in males [1]. However, in the past 10 years, the probability of cause of death from prostate cancer has increased and will increase rapidly in the future [1,2]. In the present study, we have conducted a prospective randomized control trial (RCT) for locally advanced prostate cancer in order to clarify how to treat it with adjuvant endocrine therapy after external beam radiation therapy (EBRT). The previous RCT for locally advanced prostate cancer already revealed that cancer causes of death and also all causes of death may decrease in men treated with both EBRT and endocrine therapy (neoadjuvant and/or adjuvant) in comparison with those treated with EBRT alone [3–5]. Bolla et al. [3] demonstrated that 5-year disease-free survival was higher at 85% in patients with locally advanced prostate cancer treated with EBRT and 3 years of endocrine therapy than in those treated with EBRT alone. However, the optimal timing and duration for endocrine therapy as adjuvant or neoadjuvant treatment with EBRT have not been solved. Furthermore, those issues should be discussed in terms of not only survival advantage, but also improvement of QOL.

Alternatively, the concept of intermittent endocrine therapy was proposed as a possible treatment to prolong the hormone naïve status of prostate cancer. According to basic research on androgen-dependent Shionogi carcinoma in mice, androgen-dependent status recovered after endocrine treatment was stopped in hormone-independent prostate cancer. This phenomenon would result in induction of apoptosis several times during intermittent androgen deprivation [6]. Although the treatment efficacy of intermittent hormonal therapy has not been confirmed in clinical settings, there may be some advantages in the cost for treatment, prevention of osteoporosis development, and recovery of libido.

The present assessment of combination therapy with EBRT and endocrine therapy for locally advanced prostate cancer may be of positive concern. However, it may be difficult to answer how long neoadjuvant and/or adjuvant endocrine therapy should be used. Several RCTs have been carried out or are ongoing in Europe and the USA. However, there have been no RCTs comparing the treatment efficacy and QOL between long-term adjuvant endocrine therapy and intermittent adjuvant endocrine therapy after treatment with EBRT and neoadjuvant endocrine therapy for locally advanced prostate cancer. To answer uncertainties on the above issues, the present multi-center RCT was conducted as a national cancer research project, which has been supported by the Ministry of Health, Labor and Welfare in Japan.

The primary endpoint of this study is biochemical relapse-free survival and the secondary endpoints are overall survival, cancer-specific survival and longitudinal QOL assessment between two groups. It is expected that the survival advantage by means of biochemical relapse-free survival in the continuous adjuvant endocrine treatment group may be better than that in the intermittent endocrine treatment group. Alternatively, adverse effects in patients treated with long-term androgen deprivation may increase in comparison with those treated with intermittent androgen deprivation. After completing this RCT, we expect to be able to distinguish patients who can benefit more from continuous hormonal treatment by means of survival with minimized adverse effect from those who can benefit more from intermittent hormonal treatment by means of maintaining QOL without dying of prostate cancer or suffering cancer-related complications.

MATERIALS AND METHODS

Study Protocol

Patients were eligible to participate in the protocol at any of 15 medical centers if they had biopsy-proven untreated adenocarcinoma of the prostate with clinical stage T3N0M0 or T4N0M0 (bladder neck invasion alone) and were younger than 80-years-old. Clinical stage was confirmed according to UICC 1997 by digital rectal examination (DRE), transrectal ultrasonography (TRUS), chest X-ray, bone scan, abdominal-to-pelvic CT and pelvic MRI. Patients who were treated with antiandrogen or any adrenocortical steroid hormones, or had undergone subcapsular prostatectomy or transurethral resection of the prostate including laser ablation for benign prostatic hyperplasia, were

eliminated from this study. Pelvic MRI was conducted before or 3 months after prostate biopsy.

Patients were registered as primary candidates of the study and were treated with 2 weeks of steroidal antiandrogen (chlormadinone acetate; CMA), then with both luteinizing hormone-releasing hormone (LHRH) agonist (leuprorelin or goserelin) and another 2 weeks of antiandrogen, and thereafter with LHRH agonist alone. After 6 months of endocrine treatment with LHRH agonist, only patients with PSA levels lower than 10 ng/ml, with a PSA level lower than the pretreatment level and without clinically apparent metastatic disease were enrolled in the following protocol as final candidates (2nd-line registration). All Gleason scores were reviewed by one urologic pathologist (M.H.) before the 2nd-line registration. After the 2nd-line registration was done, the patients were randomly divided into two groups according to institutions, age (younger than 70, 70 years, or older), PSA levels after 6 months of endocrine treatment (4.0 ng/ml or lower, 4.1 ng/ml or greater), and Gleason score (7 or less, 8–10) as follows: (1) continuous androgen ablation group (arm 1), (2) intermittent androgen ablation group (hormonal therapy must be stopped 6 months after the day of final EBRT treatment)

(arm 2) (Fig. 1). All of these patients were treated with EBRT immediately after completing 2nd-line registration.

Details on the procedures of radiation therapy were specified in the protocol as follows: (1) radiation field should be limited to the prostate in all cases, and the seminal vesicle should be included in radiation fields only in cases with seminal vesicle involvement being highly suspected by imaging. Elective pelvic lymph node irradiation is not performed. (2) Conformal radiation therapy, 4-field oblique or box technique, or pendulum methods are recommended in order to minimize adverse effects in the rectum and bladder. (3) A total dose of 72 Gy should be given in 36 fractions, 5 fractions per week. (4) Verification films should be taken at least two times during the radiation therapy. (5) The gross tumor volume (GTV) and clinical target volume (CTV) are the prostate gland in cases without seminal vesicle involvement. The planning target volume (PTV) margin is 10 mm from the CTV. In cases with seminal vesicle involvement, the GTV and CTV include the seminal vesicles in addition to the prostate gland. In multi-portal treatment, every portal should be irradiated in every treatment. (6) Only photon beam energy of 6 MV or more is accepted.
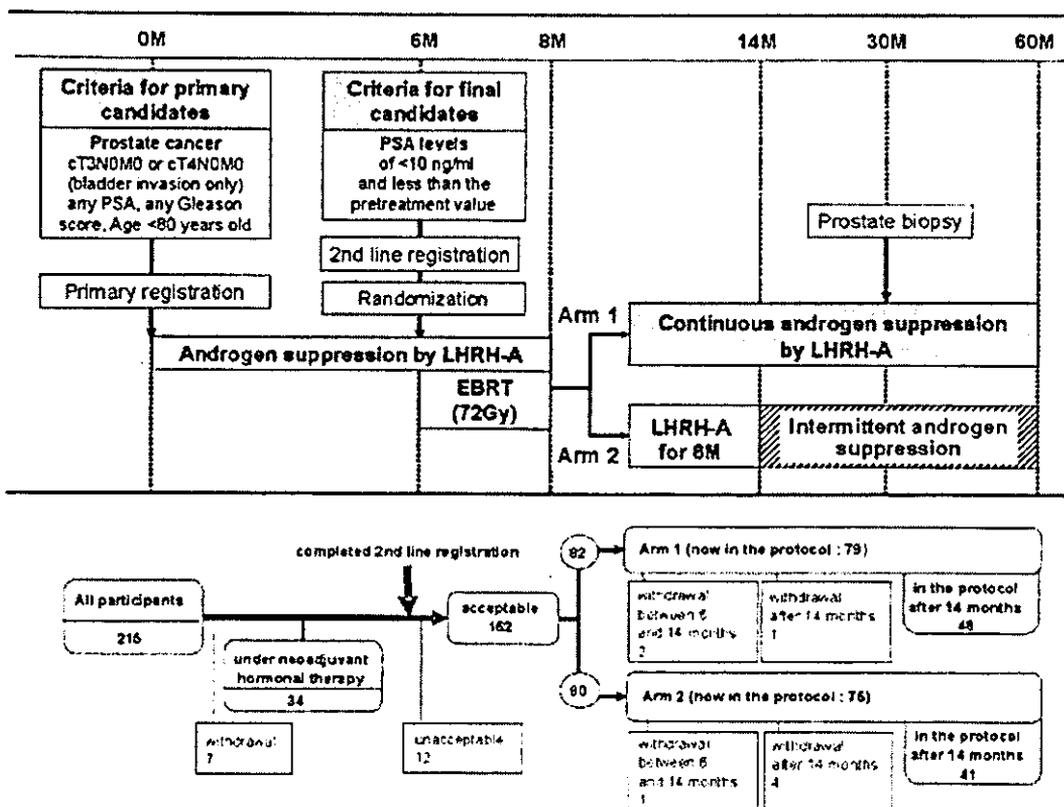

Fig. I.   Scheme of the study protocol, the number of patients registered and the present status of those patients in this study protocol. LHRH-A, LHRH agonist; EBRT, external beam radiation therapy.

Acute radiation morbidity should be evaluated by using common toxicity criteria of NCI within 90 days after radiation therapy, and late radiation morbidity should be evaluated by using the late radiation morbidity criteria of RTOG/EORTC.

Patients assigned to the intermittent androgen ablation group (arm 2) resumed hormonal therapy if they had PSA level of 10 ng/ml or greater or a clinical recurrence of disease. Resumed hormonal therapy would continue until the PSA levels decreased to below 1.0 ng/ml. If the PSA levels did not decrease to below 1.0 ng/ml, the possibility of biochemical recurrence of disease would be evaluated using the criteria in the study.

Biochemical failure was defined according to modified ASTRO criteria as follows: (1) three consecutive PSA increases in every 3-month interval and with a PSA velocity per 3 months of 0.5 ng/ml or greater, or (2) PSA levels increasing to 10 ng/ml or more. If three consecutive monthly-checked PSA levels increased rapidly at a PSA velocity per month of 0.17 ng/ml or greater, the researchers could designate that phenomenon a biochemical recurrence. The day of biochemical recurrence was defined between the day immediately before PSA increase and the day of initial PSA increase.

Clinical relapse was defined as progressive disease at a new site, an increase in the size of a nodule or cancer lesion on any images of the prostate, worse performance status, or body weight loss due to progression of prostate cancer.

Figure 2 shows the clinical assessment schedule of evaluation of treatment efficacy, QOL and adverse effects. PSA levels are measured monthly. Bone scan, abdominal-to-pelvic CT and chest X-ray must be conducted every 6 months for 1 year, and yearly

thereafter. Pelvic MRI is conducted yearly. Prostate biopsy is recommended at around 2 years after the first date of EBRT. QOL can be assessed using FACT-P and part of the UCLA prostate cancer index before the initial endocrine therapy (0 months), immediately before EBRT (6 months), immediately after EBRT (8 months), 6 months after EBRT is completed (14 months), and 6 months after dividing the patients into two arms (20 months).

In the present study, treatment efficacy, adverse effects and QOL were compared between the two groups. The primary endpoint was biochemical (PSA) relapse-free survival. The secondary endpoints were overall survival, cause-specific survival, and longitudinal QOL assessment.

Cost effectiveness was also compared between men treated with continuous endocrine therapy and those with intermittent hormonal therapy.

The study protocol of this RCT and the documents of informed consent for the participants were approved by the IRB of all facilities, and a copy of the IRB approval document has been stored in the research bureau.

Statistical Consideration on Primary Endpoint of the Study

There has been no conclusive information on the optimal treatment strategy of adjuvant endocrine therapy after EBRT in patients with locally advanced prostate cancer. Therefore, the present study was conducted on the basis of the following two hypotheses. First, there was the non-recessive hypothesis, that the cumulative biochemical relapse-free survival rate in the intermittent endocrine therapy group (arm 2) would not be remarkably worse than that in the

Variables	Months after enrollment																				
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
PSA measurement	◉					monthly check-up															◉
Digital rectal examination	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉
Transrectal ultrasonography	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉	○	◉
Abdominal and pelvic CT	◉		◉		◉				◉				◉				◉				◉
Pelvic or endorectal MRI	◉			◉					◉				◉				◉				◉
Bone scintigraphy	◉		◉		◉				◉				◉				◉				◉
Chest X-P or Chest CT	◉		◉		◉				◉				◉				◉				◉
Prostate biopsy	◉										◉										◉
QOL assessment	◉		◉	◉(8M)		◉(14M)		◉(20M)													
Uroflowmetry	○			○					○				○				○				○
Residual urine	○			○					○				○				○				○
Blood test	◉	◉	◉	◉	◉		◉		◉		◉		◉		◉		◉		◉		◉
Performance status	◉			◉					◉				◉				◉				◉

◉ Essential assessment
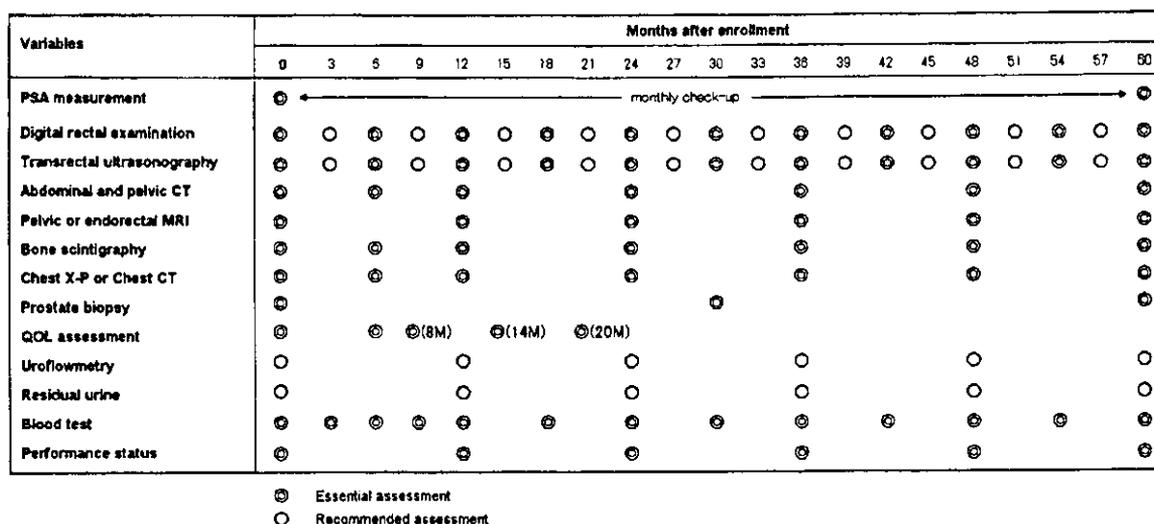○ Recommended assessment

Fig. 2. Assessment protocol for treatment effects, adverse effects and QOL in the study.

continuous endocrine therapy group (arm 1). If intermittent endocrine therapy after definitive EBRT is acceptable, the present study may be worthwhile from social, economic, and QOL points of view. The study would verify that the cumulative biochemical relapse-free survival rate in the continuous endocrine therapy group (arm 1) can be significantly better than that in the intermittent endocrine therapy group (arm 2). The second hypothesis was that continuous androgen suppression after EBRT may be worthwhile in terms of treatment efficacy, because of the specific characteristics of treatment for prostate cancer, which is famous for being hormone-naïve for a while in most cases. It would be possible to verify both of the above-mentioned hypotheses simultaneously by investigating the interval estimation of the hazard ratio, if the linearity can assume either hypothesis by carrying out the interval estimation of the hazard ratio, if the linearity can assume the recurrence hazard. Then, the 90% confidence interval for the hazard ratio (intermittent group/continuous group) can be calculated at both sides. If the upper limit is within the acceptable threshold, then the non-recessive hypothesis has been verified. On the other hand, the survival rate of the continuous group (arm 1) would be considered significantly excellent if the lower limit surpasses 1.

The main subjects for the analyses are qualified patients from whom the protocol treatments have been properly conducted. The analysis is limited to cases without remarkable contravention and deviation is carried out. The survival curve and recurrence-free survival will be estimated using Kaplan–Meier methods, and the confidence interval of the proportion at 3 and 5 years calculated by the formula of Greenwood. The hazard ratio is estimated by score statistic values from the log rank test results. Supplemental, by the hazard ratio is estimated by the Cox's proportional hazard model using the allocated factors at the 2nd registry, except for that of the facilities. The verification of the proportion hazard is done by double logarithm plotting, and the necessary analysis is carried out for the interpretation of results, such as the appliance of the Cox's proportional hazard model for time-dependent changes of the effects, when there is a remarkable dissociation from the proportion hazard. Prognostic factors which seem to be important are analyzed by means of each allocated factor at the 2nd registry except for that of the facilities, and the uniformity of differences between the two groups is examined. If necessary, the interaction between each facility and its remaining allocated factors at the 2nd registry will be analyzed, and also the differences between one facility and another.

The upper limits for the determination of non-recessiveness are 1.5 and 1.333. These upper limits may

be acceptable if the hazard for combination treatment with EBRT and long-term endocrine therapy is outlining these thresholds compared with that for EBRT alone. These consequences have already been clarified by Bolla et al. [3], in which the confidence interval of hazard for disease-free survival was demonstrated between 1/0.15 and 1/0.32. According to the results of the Bolla study [3], an upper limit for the determination of non-recessiveness of 1.5 may be acceptable. On the other hand, the upper limit of 1.333 will also be used for an alternative analysis, because it may be a reference threshold for RCTs comparing treatment efficacy for other cancers.

Intermediate Assessment and the Possibility of Withdrawal of This Protocol

At the time when the number of enrolled cases reaches half of the expected adequate number of cases, an intermediate analysis will be performed to investigate whether the main purpose of the test has already been achieved, and another at the time when the expected adequate number of cases is fully registered. The intermediate analysis will be investigated blind by one statistician (Y.O.) at the registration center of the study in Tokyo University. If the disease-free survival in one group is significantly worse than that in the other group after careful consideration of the intermediate analysis, it will be decided whether the study protocol should continue or not.

Number of Cases Required for the Study, When to Close the Registration, and the Follow-Up Period

Considering that the cumulative PSA recurrence rate within 5 years in treatment with endocrine monotherapy for locally advanced prostate cancer in Japanese was demonstrated at about 40% [7], and that in combination therapy with EBRT and endocrine therapy was demonstrated between 15 and 64% [3,4], the cumulative PSA recurrence rate within 5 years in men treated with 3 years of adjuvant endocrine therapy and EBRT, in the present study, was assumed to be 30% [3]. For non-recessive verification using a hazard ratio of 1.5 as an upper limit, 75 events are necessary in each group in order to have 80% statistical power on the basis of the alternative hypothesis, in which there is no difference in the disease-free survival rate between both groups. Alternatively, on the basis of the alternative hypothesis which uses a hazard ratio of 2, the necessary event number for the dominance verification in both groups is 55, for 80% statistical power. There may be 90–100 events in 300 patients in the protocol during 5 years of observation. Therefore, if the cumulative disease-free survival rate in the continuous endocrine group is better with a hazard ratio of 2 or

more than that in the intermittent endocrine group, it may be possible to verify the dominance with high probability, which would be 93–95% if the number of the events is 90–100. Alternatively, if the cumulative rates for disease-free survival are similar between the two groups, pursuing non-recessive verification can not be avoided. In fact, the power decreases to 61–65% if there are 90–100 events.

It is worthwhile to consider that the significance of the study is the reevaluation by meta analysis with other clinical researchers around the world, who have almost the same hypothesis for verification, when non-recessiveness and dominance can not be verified. On the other hand, it is also possible to continue registration for another few years in some cooperative facilities, because randomization to one of two arms may be permitted even in the ethics target. Furthermore, it would also be possible to conduct a multi-factorial experiment, containing the LHRH administration period as a factor, and then performing a meta analysis.

The number of expected registered cases was set at 300 and the registration period 3 years in the protocol.

Patient Characteristics Registered

Between February 2001 and November 2003, 215 patients were registered in the protocol. Table I shows the clinicopathological features of patients registered in the present study. Age ranged from 54 to 79 years (70.6 ± 5.6, mean ± SD; 72.0, median). The median PSA level at entry was 25.3 ng/ml (45.1 ± 64.3; mean ± SD). The clinical stage was T3N0M0 in 202 (94.0%) and T4N0M0 in 13 (6.0%). The Gleason score diagnosed by the central urologic pathologist was 2–6 in 32 cases (16%), 7 in 94 cases (48%), and 8–10 in 68 cases (35%).

Details in the progression of this protocol in all participants are shown in Figure 1. On November 15, 2003, 188 patients (87.4%) were still in the protocol and 27 patients (12.6%) had withdrawn from the protocol. A total of 19, 3, and 5 cases were excluded from the protocol during 0–6 months, 6–14 months, and after 14 months of the protocol treatment, respectively. Of the 27 cases excluded from the protocol, 3 cases (11%) had adverse effects, 6 cases (22%) withdrew their agreement to this protocol, 1 case (4%) had other life-threatening cancer during the protocol treatment, 4 cases (15%) had recurrence of disease, 12 cases (44%) did not meet the criteria at the 2nd registration, and 1 case (4%) was excluded from the protocol by a contravention issue.

Of the 188 cases in the protocol, 34 patients (18%) received neoadjuvant hormonal therapy between 0 and 6 months of the protocol treatment, 64 patients (34%) were treated with EBRT and adjuvant endocrine therapy between 6 and 14 months, and 90 patients

TABLE I. Clinicopathological Features at Entry

Age	
Mean ± SD	70.6 ± 5.6
Median	72
Age distribution	
54–59	7 (3.3%)
60–64	28 (13.0%)
65–69	38 (17.7%)
70–74	80 (37.2%)
75–79	62 (28.8%)
PSA level (ng/ml)	
Mean ± SD	45.1 ± 64.3
Median	25.3
PSA distribution	
0.0–4.0	3 (1.4%)
4.1–10.0	38 (17.7%)
10.1–20.0	41 (19.1%)
20.1–50.0	79 (36.7%)
50.1–100.0	33 (15.3%)
100.1–∞	21 (9.8%)
Gleason score by (hospital pathologists)	
2–6	26 (12.1%)
7	106 (49.3%)
8–10	83 (38.6%)
Primary Gleason grade (hospital pathologists)	
–3	92 (42.8%)
4–5	123 (57.2%)
Clinical stage	
T3N0M0	202 (94.0%)
T4N0M0	13 (6.0%)
Gleason score by (central pathologist)	
2–6	32 (16.5%)
7	94 (48.5%)
8–10	68 (35.1%)
Primary Gleason grade (central pathologist)	
–3	99 (51.0%)
4–5	95 (49.0%)

(48%) were treated with continuous or intermittent androgen ablation after 14 months of the protocol treatment.

Of the 95 cases who continued the protocol treatment after 14 months, 49 were treated with continuous endocrine treatment (arm 1) and 46 were treated with intermittent endocrine treatment (arm 2). The mean follow-up duration was 22.2 months (ranged from 14 to 30 months) in arm 1 and 23.0 months (ranged from 14 to 30 months) in arm 2. Of the 49 patients registered in arm 1, 1 case (2.0%) was excluded from the protocol because of recurrence of disease. Of the 46 cases registered in arm 2, 4 cases (8.7%) were excluded from the protocol treatment, because of recurrence of disease in 2 cases, contravention of the protocol in 1 case, and their own decision in 1 case.

RESULTS

Changes in the PSA levels within 1 month before prostate biopsy (pretreatment), after 6 months of endocrine treatment, 8 months of endocrine treatment (immediately after EBRT), and 14 months of endocrine treatment (6 months after EBRT) are shown in Table II. The PSA levels showed a remarkable decrease to median (mean ± SD) levels of 1.1 ng/ml (2.7 ± 5.0), 0.2 ng/ml (0.6 ± 1.0) and 0.1 ng/ml (0.3 ± 0.5) after 6, 8, and 14 months of the protocol treatment, respectively. The proportion of patients with PSA levels of 1.0 ng/ml or lower was 49% (85/173), 81% (118/145), and 91% (86/95) at 6, 8, and 14 months of the protocol treatment.

Of the 157 cases treated with EBRT, excluding eliminated cases without recurrence of disease, 153 cases (97.5%) had no biochemical failure in the mean follow-up of 17.3 months (range from 6.7 to 34.3 months).

A total of 44 cases were treated by intermittent hormonal therapy. Of the 44 cases, 41 cases have had no endocrine treatment according to the criteria after 14 months of the protocol treatment. Of the 401 months of the post-intermittent phase (i.e., after 14 months in the protocol treatment), in all 44 cases, 394 months (98.3%) were without treatment with endocrine therapy according to the criteria (off-treatment).

Of the 44 cases within the intermittent treatment protocol, 3 cases (6.8%) resumed endocrine therapy, because of clinical progression in 1 case and PSA levels increasing to greater than 10 ng/ml in 2 cases.

DISCUSSION

Although the treatment efficacy of intermittent endocrine therapy has not been clarified, it would be expected to have significance in the QOL, cost and prevention of decreasing bone mineral density. Several investigators have demonstrated the possibility of the clinical utility of intermittent endocrine therapy. The proportion of off-treatment periods were 38–50% during 24–30 months of follow-up periods in men with prostate cancer treated with endocrine mono-therapy [8–10]. Most of the non-randomized trials have reported a response to the reintroduction of hormonal therapy in 90% of patients, with an on-treatment/off-treatment ratio of about 40–60% [8,11–17]. However, there had been no RCT to investigate the possibility of intermittent endocrine therapy in combination with EBRT in men with locally advanced prostate cancer. The biochemical recurrence rate may be higher in men treated with intermittent endocrine therapy than in those with continuous endocrine therapy. However, additional EBRT may improve disease-free survival for men with locally advanced prostate cancer. The present study revealed that the on-treatment/off-treatment ratio was extremely low at 1.8%. Therefore, the present RCT can solve uncertainties of treatment efficacy and QOL for intermittent endocrine therapy in combination with EBRT for men with locally advanced prostate cancer.

In the present study, disease-free survival was defined as a primary endpoint, because a previous study demonstrated a high 5-year overall survival rate of 92% and a relatively low 5-year biochemical disease-free survival rate of 61% in patients with locally advanced prostate cancer treated with LHRH agonist alone [7]. To set biochemical disease-free survival as the primary endpoint, it may be possible to have enough statistical power during a 5-year follow-up. The validity of this setting may be acceptable, because there is a limitation to the treatment after developing hormone-insensitive prostate cancer. Furthermore, any endocrine treatments will not be effective after recurrence of disease and the life span may be limited.

TABLE II. Changes in the PSA Levels After 6, 8, and 14 Months of the Protocol Treatment

	0 month	6 months	8 months	14 months
n	215	173	145	95
PSA level (ng/ml)				
Mean ± SD	45.1 ± 64.3	2.7 ± 5.0	0.6 ± 1.0	0.3 ± 0.5
Median	25.3	1.1	0.2	0.1
PSA distribution				
0.0–1.0	0 (0.0%)	85 (49.1%)	118 (81.4%)	86 (90.5%)
1.1–2.0	0 (0.0%)	29 (16.8%)	14 (9.7%)	9 (9.5%)
2.1–4.0	3 (1.4%)	33 (19.1%)	11 (7.6%)	0 (0.0%)
4.1–10.0	38 (17.7%)	15 (8.7%)	2 (1.4%)	0 (0.0%)
10.1–20.0	41 (19.1%)	6 (3.5%)	0 (0.0%)	0 (0.0%)
20.1–50.0	79 (36.7%)	5 (2.9%)	0 (0.0%)	0 (0.0%)
50.1–100.0	33 (15.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
100.1–∞	21 (9.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

The rates of biochemical no evidence of disease (bNED) control for patients with stage T3/T4 disease treated with a conventional dose of radiation therapy alone are poor, between 25 and 32% at 5 years [18,19] and 37% at 6 years [20]. The 5-year bNED in patients treated with EBRT alone for stage T1 to T4 disease decreased as pretreatment PSA levels increased, that is a bNED of 82–100%, 44–66%, 27–72%, and 11–14% for patients with pretreatment PSA levels of 4 ng/ml or less, 4–10 ng/ml, 10–20 ng/ml, and greater than 20 ng/ml, respectively [18,20–22]. The bNED control rate is higher in men treated with 3DCRT than in those treated with conventional EBRT even for cases with high levels of PSA. However, the bNED at 5 years is still low at 75 and 32% in patients treated with a high radiation dose of 76 Gy, in the PSA range of 10–20 ng/ml and greater than 20 ng/ml, respectively [23]. These treatment failures might result from the limitation of EBRT for large volume cancer on one side and the existence of clinically undetectable metastasis on the other side.

These poor outcomes of EBRT for locally advanced prostate cancer led to several randomized controlled trials on the effectiveness of neoadjuvant or adjuvant hormonal therapy in comparison with EBRT alone by the Radiation Therapy Oncology Group (RTOG) and The European Organization for Research and Treatment of Cancer (EORTC).

The RTOG 86-10 was conducted to investigate the usefulness of androgen ablation 2 months before and during EBRT compared with EBRT alone for locally advanced prostate cancer [5]. The biochemical disease-free survival and cause-specific mortality were significantly better in men undergoing androgen ablation before and during EBRT than in those treated with EBRT alone, especially in patients with Gleason 2–6 tumors.

Bolla et al. [3] conducted an RCT comparing overall survival and the disease-free interval between men treated with EBRT alone and with EBRT in combination with 3 years of adjuvant endocrine therapy starting from the initial date of EBRT (EORTC 22863) [3]. They demonstrated that the 5-year overall survival rate was significantly higher at 79% in patients treated with combination therapy than that in those treated with EBRT alone, which was 62%. The 5-year disease-free survival rate was also significantly higher at 81% in patients treated with combination therapy than that in those treated with EBRT alone.

The effectiveness of adjuvant endocrine therapy in combination with EBRT for patients with locally advanced prostate cancer can be clarified. Although cancer volume may be a very important factor in the treatment of EBRT, clinical data addressing the potential value of hormonal cytoreduction before radiotherapy have been quite limited. Therefore, it

can also be valuable to investigate whether neoadjuvant endocrine therapy before EBRT is useful for locally advanced prostate cancer. In the present study protocol, all patients were initially treated with endocrine therapy for 6 months, and only patients with PSA levels after 6 months of endocrine therapy of 10 ng/ml or lower and also lower than the pretreatment levels were enrolled as final candidates in this study. The eliminated cases without sufficient effects after 6 months of endocrine treatment should be treated with other treatment protocols like chemoendocrine treatment. Therefore, our study protocol, which selects only patients with sufficient effects by neoadjuvant endocrine treatment, may be acceptable by means of ethical issues and also scientific validity.

At present, EBRT in combination with adjuvant endocrine therapy for locally advanced prostate cancer can be recommended in terms of survival benefit. However, it has not been clarified when and how long additional endocrine therapy should be conducted with respect to not only survival but also QOL. The compliance of this RCT may be high, so it is expected that long-term follow-up of the participants in the present study will reveal the possibilities of intermittent endocrine therapy after EBRT in patients with locally advanced prostate cancer.

Members of the National Research Project on Endocrine-Radiation CombinationTherapy for Locally Advanced Prostate Cancer

Hidetoshi Yamanaka, Hiroyuki Fujimoto, Naoki Matsuoka, Taiji Tsukamoto, Iwao Fukui, Seiji Naito, Michiyuki Usami, Kazuhiro Suzuki, Norio Mitsuhashi, Tetsuo Akimoto, Minako Sumi, Masato Hareyama, Takashi Yamashita, Katsumasa Nakamura, Koichi Tokuuye, Kinji Nishiyama, Yasuo Ohashi, Masaoki Harada, Jun Aoki, Mitsuru Shinohara, Katsuyuki Karasawa, Satoshi Kitahara, Miwako Nozaki, Shin Egawa, Iku Nishiguchi, Masashi Kitano, Takanori Suzuki, Nobuaki Shimizu, Yoshio Tamaki, Mikio Kobayashi, Iku Takahashi, Mikinobu Ohtani, Akio Iwasaki, Tatsuo Tochigi, Shiro Saito, Katsuyoshi Hashine, Tadao Kakizoe, Toshihiko Kotake.

Associate Researchers in the Project

Kazuto Ito, Naoya Masumori, Norio Meguro, Hirofumi Koga, Sadafumi Kawamura, Kotaro Gomi, Yutaka Takezawa, Atsushi Yamauchi, Takumi Yamamoto, Akira Irie, Kazumi Shiojima.

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Featured Article

The Influence of Androgen Deprivation Therapy on Dihydrotestosterone Levels in the Prostatic Tissue of Patients with Prostate Cancer

Tsutomu Nishiyama,[1] Yutaka Hashimoto,[2] and Kota Takahashi[1]

[1]Division of Urology, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata; and [2]Pharmaceuticals Group, Nippon Kayaku Co., Ltd., Tokyo, Japan

ABSTRACT

Purpose: The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue is not clearly known. Changes in dihydrotestosterone levels in the prostatic tissue during androgen deprivation therapy in the same patients have not been reported. We analyzed dihydrotestosterone levels in prostatic tissue before and after androgen deprivation therapy.

Experimental Design: A total of 103 patients who were suspected of having prostate cancer underwent prostatic biopsy. Sixty-nine patients were diagnosed as having prostate cancer whereas the remaining 34 were negative. Serum samples were collected before biopsy or prostatectomy. Dihydrotestosterone levels in prostatic tissue and serum were analyzed using liquid chromatography/electrospray ionization-mass spectrometry after polar derivatization. In 30 of the patients with prostate cancer, dihydrotestosterone levels in prostatic tissue were determined by performing rebiopsy or with prostate tissues excised after 6 months on androgen deprivation therapy with castration and flutamide.

Results: Dihydrotestosterone levels in prostate tissue after androgen deprivation therapy remained at ~25% of the amount measured before androgen deprivation therapy. Dihydrotestosterone levels in serum decreased to ~7.5% after androgen deprivation therapy. The level of dihydrotestosterone in prostatic tissue before androgen deprivation therapy was not correlated with the serum level of testosterone. Serum levels of adrenal androgens were reduced to ~60% after androgen deprivation therapy.

Conclusions: The source of dihydrotestosterone in prostatic tissue after androgen deprivation therapy involves intracrine production within the prostate, converting adrenal androgens to dihydrotestosterone. Dihydrotestosterone still remaining in prostate tissue after androgen deprivation therapy may require new therapies such as treatment with a combination of 5α-reductase inhibitors and antiandrogens, as well as castration.

INTRODUCTION

Since the observation of Huggins and Hodges (1) that disseminated prostate cancer reacts favorably to castration or the administration of estrogenic hormones, first-line hormonal therapy has been used to impair the production or activity of androgens or both.

Testosterone is converted to dihydrotestosterone by 5-α reductase in the prostate. There have been several reports that examined in detail the method for quantitative analysis of the tissue dihydrotestosterone concentrations of the prostate (2–5). Belanger *et al.* (5) and Labrie *et al.* (6) stated that after the elimination of testicular androgens, the intraprostatic concentration of dihydrotestosterone remains at ~40%. These data indicate that a substantial level of dihydrotestosterone remains in the prostate after castration. Belanger *et al.* (5) and Labrie *et al.* (6) also suggested that dihydrotestosterone completely disappears from the prostate after androgen deprivation therapy with castration and flutamide. The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue in prostate cancer, however, is not fully known. Changes in dihydrotestosterone levels in the prostatic tissue during androgen deprivation therapy for prostate cancer in the same patients have not been reported. One of the reasons is that the detectable quantity of dihydrotestosterone involved in the prostatic tissue collected from needle biopsy samples is minute. We, however, have developed a detection system for minuscule quantities of dihydrotestosterone with liquid chromatography/electrospray ionization-mass spectrometry after polar derivatization of dihydrotestosterone (7).

Therefore, we analyzed dihydrotestosterone levels in prostatic tissue and endogenous hormone levels in serum both in patients with prostate cancer and those without prostate cancer who underwent prostatic biopsy. The patients diagnosed with clinically localized prostate cancer, furthermore, were treated with androgen deprivation therapy in a neoadjuvant setting for 6 months. We then carried out rebiopsy or prostatectomy 6 months after androgen deprivation therapy treatment to analyze dihydrotestosterone levels in prostatic tissue and endogenous hormone levels in serum.

PATIENTS AND METHODS

Patients. Between April 2000 and October 2002, 103 patients suspected of having prostate cancer underwent prostatic biopsy. Those patients diagnosed with clinically localized prostate cancer were given androgen deprivation therapy (castration and flutamide) in a neoadjuvant setting for 6 months. Baseline patients' characteristics are listed in Table 1. This research was reviewed and approved by the Institutional Review Board. Written informed consent was obtained from all participants.

Sample Collections. To determine dihydrotestosterone levels, the samples of prostatic tissue were obtained from the midlateral region of the prostate with a 16-gauge biopsy needle; alternatively, prostatectomy specimens were used. Serum samples for endocrine study were collected from the patients between 9:00 and 12:00 p.m. (noon). In all patients who underwent ultrasound-guided biopsy or radical prostatectomy, serum samples were obtained before the respective interventions. Serum samples were stored at $-20°C$ until additionally processed. All biopsies and prostatectomy specimens were analyzed by conventional pathological examination. Tissue samples were stored at $-80°C$ until additional processing.

Hormones and Prostate-specific Antigen Levels of Serum Samples Other Than Dihydrotestosterone. The prostate-specific antigen and hormones were quantified by commercially available immunoassays: prostate-specific antigen [TOSOH-II (PA)], luteinizing hormone, and follicle-stimulating hormone. All hormones were quantified by automated fluorescence polarization assays on Tosoh equipment (Tosoh Corporation, Tokyo, Japan). Serum levels of testosterone, adrenocorticotropic hormone (ACTH), cortisol, androstenedione, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), and prolactin were determined by radioimmunoassay (BML, Tokyo, Japan).

Sensitive Analysis of dihydrotestosterone in Prostatic Tissues and Serum Samples by Semi–Micro-Liquid Chromatography/Electrospray Ionization-Mass Spectrometry after Polar Derivatization. The dihydrotestosterone levels in prostatic tissue and serum were analyzed by liquid chromatography/electrospray ionization-mass spectrometry after polar derivatization of dihydrotestosterone, as described previously (7). The polar derivatization method for electrospray

ionization was developed and applied to the sensitive analysis of dihydrotestosterone. Dihydrotestosterone in prostatic tissue was dissolved in alkaline solution and extracted via a solid-phase column and derivatized to N-methylpyridinium-dihydrotestosterone as a polar derivative. N-Methylpyridinium-dihydrotestosterone was purified by Bond Elut C18 and determined with a semi–micro-liquid chromatography/electrospray ionization-mass spectrometry with selected reaction monitoring. The calibration graph was linear from 5 to 100 pg/tube. The lowest dihydrotestosterone level in this method was 5 pg/tube.

Statistical Analysis. Statistical comparison of hormonal levels in patients with prostate cancer before treatment and noncancer patients at diagnosis was carried out with the Mann-Whitney U test. Statistical comparison of DHEA level in patients with prostate cancer before treatment and noncancer patients at diagnosis was also carried out with a multivariate analysis with logistic regression after forcing age in the model. Statistical comparison of the influence of androgen deprivation therapy on hormonal levels was carried out with the Wilcoxon's signed rank test. The correlation between the dihydrotestosterone levels or ACTH and ACTH or other androgens was analyzed with the Spearman rank correlation coefficient. The test was two-sided, and a P value of <0.05 was considered statistically significant. Statistical analyses were carried out with SPSS software v.11.0 for PC (SPSS, Inc., Chicago, IL).

RESULTS

Clinical Results. Sixty-nine patients were diagnosed as having prostate cancer and 34 as having a nonmalignant prostate condition. The patients' characteristics are listed in Table 1. Thirty of the 69 patients were treated with androgen deprivation therapy with a luteinizing hormone-releasing hormone agonist (goserelin acetate or leuprolide acetate) or bilateral orchiectomy and flutamide in a neoadjuvant setting for 6 months. Eight of the 30 patients were withdrawn from flutamide treatment because of adverse effects during the following dosing periods: 1 month, 1 patient; 2 months, 1 patient; 4 months, 3 patients; and 5 months, 3 patients. Six patients were withdrawn because of liver dysfunction and two because of diarrhea.

Table 1 Patient characteristics

	Total	With cancer	Without cancer
No. of patients	103	69	34
Age (y) at diagnosis [mean (range)]	69 (41–86)	71 (45–86)*	66 (41–81)*
PSA [ng/mL, median (range)]	14.9 (3.0–19578)	27.4 (4.7–19578)†	8.6 (3.0–27.8)†
Gleason score [mean (range)]		6 (4–10)	
M0		54	
M1		15	
Androgen deprivation therapy		30	
Age (y) at diagnosis [mean (range)]		71 (57–78)	
LH-RHa + flutamide		25	
Castration + flutamide		5	

Abbreviations: LH-RHa, luteinizing hormone-releasing hormone agonist.
* $P = 0.004$.
† $P < 0.001$.

Dihydrotestosterone Levels in Prostatic Tissue and Serum Hormone Levels in Patients with Prostate Cancer ($n = $ 69) before Treatment and Noncancer Patients ($n = $ 34) at Diagnosis. In this study, the serum DHEA level was significantly lower in patients with prostate cancer by comparison with noncancer patients using the Mann-Whitney U test ($P = 0.037$; Table 2). However, there is no statistical association between prostate cancer and DHEA level using results of the logistic regression model ($P = 0.762$). There were no statistically significant differences between the patients with prostate cancer and the patients without prostate cancer in LH ($P = 0.165$), follicle-stimulating hormone ($P = 0.206$), prolactin ($P = 0.169$), ACTH ($P = 0.788$), cortisol ($P = 0.770$), testosterone ($P = 0.539$), androstenedione ($P = 0.509$), and DHEA-S ($P = 0.404$), including dihydrotestosterone levels in serum ($P = 0.602$) and prostatic tissue ($P = 0.302$) in this study. There were no statistically significant differences between the patients with prostate cancer and the patients without prostate cancer with respect to the ratios of testosterone to serum dihydrotestosterone ($P = 0.772$) and dihydrotestosterone in prostatic tissue ($P = 0.191$).

Correlation between the Dihydrotestosterone Levels and Other Androgens in Patients with Prostate Cancer before Treatment and Noncancer Patients at Diagnosis ($n = $ 103). The level of dihydrotestosterone in prostatic tissue before androgen deprivation therapy was not correlated with the serum level of testosterone (rs $= 0.010$, $P = 0.923$; Table 3). The level of dihydrotestosterone in prostatic tissue was correlated with the serum levels of DHEA (rs $= 0.243$, $P = 0.014$) and DHEA-S (rs $= 0.239$, $P = 0.015$). There was a small correlation between the serum level of dihydrotestosterone and the level of dihydrotestosterone in prostatic tissue (rs $= 0.229$, $P = 0.025$, y $= 0.001$x $+ 5.0165$). The serum level of dihydrotestosterone was correlated with the serum levels of testosterone (rs $= 0.425$, $P < 0.001$) and DHEA (rs $= 0.305$, $P = 0.003$).

The Influence of Androgen Deprivation Therapy [Total ($n = 30$), 6 Months with Flutamide ($n = 22$), and Flutamide Withdrawal ($n = 8$)] on Hormone Levels. The serum levels of ACTH ($P < 0.001$), testosterone ($P < 0.001$), androstenedi-

Table 3 The correlation between the DHT levels and other androgens in patients with prostate cancer and noncancer patients at diagnosis ($N = 103$)

	sDHT	tDHT
Testosterone	rs $= 0.425$	rs $= 0.010$
	$P < 0.001$	$P = 0.923$
Androstenedione	rs $= 0.254$	rs $= 0.019$
	$P = 0.130$	$P = 0.852$
DHEA	rs $= 0.305$	rs $= 0.243$
	$P = 0.003$	$P = 0.014$
DHEA-S	rs $= 0.065$	rs $= 0.239$
	$P = 0.530$	$P = 0.015$
sDHT		rs $= 0.229$
		$P = 0.025$
tDHT	rs $= 0.229$	
	$P = 0.025$	

Abbreviations: sDHT, dihydrotestosterone level in serum; tDHT, dihydrotestosterone level in prostatic tissue.

one ($P < 0.001$), DHEA ($P = 0.001$), DHEA-S ($P < 0.001$), and dihydrotestosterone ($P < 0.001$) and the level of dihydrotestosterone in prostatic tissue ($P < 0.001$) significantly declined after androgen deprivation therapy (Table 4). The dihydrotestosterone levels in prostatic tissue after androgen deprivation therapy, however, remained at ~25% of those measured before androgen deprivation therapy. The dihydrotestosterone levels in serum decreased to ~7.5% after androgen deprivation therapy. Testosterone levels decreased to ~2.7% after androgen deprivation therapy, and serum hormone levels were reduced to 59% for ACTH, 52% for androstendione, 60% for DHEA, and 64% for DHEA-S. The decrease in adrenal androgens in the flutamide withdrawal cases was less significant than that in the flutamide cases. The prolactin level ($P = 0.737$) and cortisol level ($P = 0.148$) in serum did not decline after androgen deprivation therapy.

Correlation between the Dihydrotestosterone Levels or ACTH and ACTH or Other Androgens after androgen deprivation therapy. The level of dihydrotestosterone in prostatic tissue was correlated with the serum level of testosterone (rs $= 0.390$, $P = 0.033$; Table 5). The level of dihydrotestos-

Table 2 Pretreatment serum hormones

	Patients with cancer Mean (SD)	Patients without cancer Mean (SD)	P	Logistic regression analysis P	Odds ratio	95% confidence interval
Age (y)	71 (45–86)	66 (41–81)	0.004	0.015	0.928	0.874–0.986
LH (mIU/mL)	6.6 (5.8)	4.61 (2.5)	0.165			
FSH (mIU/mL)	20.0 (19.4)	12.8 (6.0)	0.206			
PRL (ng/mL)	10.5 (18.4)	7.0 (3.4)	0.169			
ACTH (pg/mL)	42.7 (34.2)	44.3 (34.7)	0.788			
Cortisol (μg/dL)	15.3 (5.5)	15.6 (4.6)	0.770			
Testosterone (ng/dL)	449.3 (170.5)	425.0 (133.0)	0.539			
Androstene dione (ng/mL)	0.81 (0.41)	0.86 (0.41)	0.509			
DHEA (ng/mL)	1.79 (1.26)	2.26 (1.35)	0.037	0.762	1.058	0.734–1.524
DHEA-S (ng/mL)	1169.8 (803.3)	1263.0 (876.4)	0.404			
sDHT (pg/mL)	462.5 (274.6)	423.9 (243.2)	0.602			
tDHT (ng/g tissue)	5.19 (2.50)	5.61 (1.96)	0.302			
Testosterone/sDHT	1.27 (1.00)	1.07 (0.59)	0.772			
Testosterone/tDHT	99.5 (67.8)	78.9 (44.5)	0.191			

Abbreviations: sDHT, dihydrotestosterone level in serum; tDHT, dihydrotestosterone level in prostatic tissue.

Table 4 The influence of ADT [total (N = 30), 6 months with flutamide (N = 22), and flutamide withdrawal (N = 8)] on hormone levels

	Before ADT Mean (SD)	After ADT (N = 30) Mean (SD) P	6 months with flutamide (N = 22) Mean (SD) P	Flutamide withdrawal (N = 8) Mean (SD) P
PRL (ng/mL)	8.2 (4.0)	7.6 (2.3) 0.737	8.2 (2.3) 0.709	8.4 (5.3) 0.208
ACTH (pg/mL)	48.3 (46.0)	28.3 (12.1) <0.001	28.2 (13.7) 0.009	28.4 (6.2) 0.327
Cortisol (μg/dL)	15.3 (4.5)	15.6 (5.2) 0.148	15.9 (4.5) 0.182	13.7 (5.5) 0.715
Testosterone (ng/dL)	460.8 (192.4)	12.4 (6.8) <0.001	10.4 (5.4) <0.001	18.0 (7.6) 0.012
Androstenedione (ng/mL)	0.81 (0.36)	0.42 (0.22) <0.001	0.38 (0.21) <0.001	0.52 (0.24) 0.025
DHEA (ng/mL)	2.03 (1.32)	1.22 (0.76) 0.001	1.06 (0.56) 0.001	1.64 (1.09) 0.484
DHEA-S (ng/mL)	1194.9 (855.0)	761.3 (875.6) <0.001	654.7 (505.7) <0.001	1054.0 (994.9) 0.123
sDHT (pg/mL)	503.4 (315.9)	38.0 (31.2) <0.001	33.0 (27.0) <0.001	51.8 (39.3) 0.012
tDHT (ng/g tissue)	5.44 (2.84)	1.35 (1.32) <0.001	1.23 (1.47) <0.001	1.69 (0.77) 0.036

Abbreviations: ADT, androgen deprivation therapy; sDHT, dihydrotestosterone level in serum; tDHT, dihydrotestosterone level in prostatic tissue.

terone in prostatic tissue was not correlated with the serum level of androgens other than testosterone. The serum level of dihydrotestosterone was correlated with the serum levels of androstenedione (rs = 0.466, P = 0.009), DHEA (rs = 0.577, P = 0.001), and DHEA-S (rs = 0.480, P = 0.007). There was no correlation between the serum level of dihydrotestosterone and the level of dihydrotestosterone in prostatic tissue (rs = 0.013, P = 0.869). There was no correlation between the serum level of ACTH and the serum levels of androgens and the level of dihydrotestosterone in prostatic tissue.

DISCUSSION

Our results showed that after androgen deprivation therapy with castration and flutamide, the dihydrotestosterone level in prostatic tissue remained at ~25% of the amount measured before androgen deprivation therapy in the same patients. Previous reports revealed that the mean dihydrotestosterone levels in the prostate tissue treated with androgen deprivation therapy were between 10 and 40% of those of untreated prostrate tissue (2–5). Mohler *et al.* (8) showed that the dihydrotestosterone level in recurrent prostate cancer tissue was decreased to 18% of the level in benign prostate tissue. Belanger *et al.* (5) and Labrie *et al.* (6) indicated that androgen deprivation therapy with castration and flutamide decreases intraprostatic dihydrotestosterone to the point where it is undetectable. Our data, however, indicates that flutamide acts to suppress the binding of the residual dihydrotestosterone to androgen receptors, not to decrease intraprostatic dihydrotestosterone to undetectable levels.

It is not clear to what extent the testosterone and dihydrotestosterone in prostate tissue derives from adrenal androgens or other steroid precursors. Previous reports showed that persistent levels of prostatic dihydrotestosterone after castration are derived from adrenal androgens in the prostate (3, 5, 8). A sulfatase is present in human prostate that converts DHEA-S to

DHEA (9). The plasma concentration of DHEA-S is 100 to 500 times higher than that of testosterone. Koh *et al.* (10, 11) revealed that prostate cancer cells have the ability to convert adrenal androgens to dihydrotestosterone intracellularly. Mohler *et al.* (8) revealed that recurrent prostate cancer tissue levels of adrenal androgens were ~50% the levels in benign prostate. In our data, the level of dihydrotestosterone in prostatic tissue before androgen deprivation therapy was not correlated with the serum level of testosterone (Table 3). The level of dihydrotestosterone in prostatic tissue after androgen deprivation therapy was only correlated with the serum level of testosterone (Table 5). The level of dihydrotestosterone in prostatic tissue before androgen deprivation therapy was correlated with the serum level of adrenal androgens other than androstenedione (Table 3). The serum dihydrotestosterone level after androgen deprivation

Table 5 The correlation between the DHT levels or ACTH and ACTH or other androgens after androgen deprivation therapy (N = 30)

	sDHT	tDHT	ACTH
ACTH	rs = 0.103 P = 0.586	rs = 0.347 P = 0.060	
Testosterone	rs = 0.260 P = 0.165	rs = 0.390 P = 0.033	rs = −0.014 P = 0.942
Androstenedione	rs = 0.466 P = 0.009	rs = 0.351 P = 0.057	rs = 0.326 P = 0.079
DHEA	rs = 0.577 P = 0.001	rs = 0.071 P = 0.708	rs = 0.080 P = 0.674
DHEA-S	rs = 0.480 P = 0.007	rs = 0.341 P = 0.065	rs = 0.017 P = 0.930
sDHT		rs = −0.013 P = 0.869	rs = 0.103 P = 0.586
tDHT	rs = −0.013 P = 0.869		rs = 0.347 P = 0.060

Abbreviations: sDHT, dihydrotestosterone level in serum; tDHT, dihydrotestosterone level in prostatic tissue.

therapy was correlated with serum levels of adrenal androgen (Table 5). These findings suggest that serum testosterone after androgen deprivation therapy mostly comes from adrenal androgens converted in the prostatic cells. These findings could also suggest that serum dihydrotestosterone after androgen deprivation therapy comes from adrenal androgens converted in the peripheral tissues, including the prostate. It is possible that the prostate is the major dihydrotestosterone-producing organ, and the level of dihydrotestosterone in prostatic tissue is correlated with the level of adrenal androgens and testosterone in prostatic tissue. These results reveal that the source of dihydrotestosterone in prostatic tissue after androgen deprivation therapy involves intracrine production within the prostate to convert adrenal androgens to dihydrotestosterone.

The serum hormone levels were reduced to ~60% in ACTH, androstenedione, DHEA, and DHEA-S after androgen deprivation therapy with castration and flutamide in our study. The mechanism causing the decrease of adrenal androgens after androgen deprivation therapy has yet to be determined (12–14). Several investigators have shown the effects of flutamide on the plasma levels of adrenal androgens (12, 14). Flutamide allegedly decreases adrenal androgens after treatment by castration and flutamide (13, 14). Our results also showed that castration and flutamide reduced adrenal androgens to ~60%. The serum ACTH level after androgen deprivation therapy was not correlated with serum adrenal androgen levels and dihydrotestosterone levels in serum and prostatic tissue. Prolactin and cortisol in serum did not decline after androgen deprivation therapy. The mechanism of the suppression of adrenal androgens is speculated to be by flutamide having an inhibitory effect on human adrenal microsomal 17α-hydroxylase and 17,20-lyase activities (14).

A recent collaborative meta-analysis has shown that the addition of a nonsteroidal antiandrogen (flutamide or nilutamide) to castration reduced highly significantly the risk of death (all causes of death) by 8% (95% confidence interval, 3–13; $P = 0.005$), which translates to a small but significant improvement in 5-year survival of 2.9% over castration alone (15). Most meta-analyses show a positive result with nonsteroidal antiandrogens (16). The percentage of PSA responses has been shown to be significantly higher among patients receiving androgen deprivation therapy composed of castration and flutamide than among patients undergoing castration only (17). Labrie et al. (18) showed long-term and continuous androgen deprivation therapy could offer the possibility of long-term control or possible cure of localized prostate cancer. It is established that 5α-reductase inhibitor such as finasteride can reduce intraprostatic levels of dihydrotestosterone (19). Visakorpi et al. (20) showed that amplification of the androgen receptor gene is increased during androgen deprivation therapy. Gregory et al. (21) showed that the androgen receptor is transcriptionally active in recurrent prostate cancer and can increase cell proliferation at the low levels of androgen that occur after androgen deprivation therapy. Zegarra-Moro et al. (22) revealed that therapies that target the androgen receptor directly with an androgen receptor antibody or androgen receptor ribozymes inhibited growth of both androgen-sensitive and androgen-refractory prostate cancer in vitro. Chen et al. (23) revealed that the increase in androgen receptor mRNA and protein was both

necessary and sufficient to convert prostate cancer growth from a hormone-sensitive to a hormone-refractory stage and was dependent on a functional ligand-binding domain. Increased levels of androgen receptor confer resistance to antiandrogens by amplifying signal output from low levels of residual ligand and by altering the normal response to antagonists (23). Leibowitz and Tucker (24) revealed that triple androgen blockade therapy followed by finasteride maintenance appears to be a promising alternative for the management of patients with clinically localized or locally advanced prostate cancer. These findings and our results suggest that new therapies that target androgen receptor and prevent formation of androgens within prostate cancer cells such as treatment with a combination of antiandrogens and 5α-reductase inhibitors can block the stimulation from adrenal androgens that contributes ~25% of total dihydrotestosterone when they are combined with testicular suppression of androgens and may offer the most effective androgen deprivation therapy to prolong remission of prostate cancer as of now.

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