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Original Article: Clinical Investigation

Naftopidil versus tamsulosin hydrochloride for lower urinary tract symptoms associated with benign prostatic hyperplasia with special reference to the storage symptom: A prospective randomized controlled study

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Objectives: In order to compare the clinical efficacy of naftopidil (Naf) and tamsulosin hydrochloride (Tam), which differ in their selectivity to alpha receptor subtypes, we performed a multi-center prospective randomized controlled study.

Methods: Men complaining of lower urinary tract symptoms due to benign prostatic hypertrophy, were randomized into two treatment groups: one receiving 50 mg Naftopidil daily (Naf group, $n = 31$ pts), and one receiving 0.2 mg Tam once daily (Tam group, $n = 28$ pts). Baseline symptom scores were compared to those at 2 weeks and at the end of the observation period (6–8 weeks).

Results: In the Naf group at 2 weeks, the score of the daytime frequency significantly improved from 3.5 to 2.2 ($P = 0.03$), and the score of nocturia improved significantly from 3.5 to 2.2 ($P = 0.0004$), respectively. In the Tam group at 2 weeks, however, no significant improvement was noted in the increased score of daytime frequency ($P = 0.1$) or nocturia ($P = 0.2$). At 2 weeks, the storage symptom score of the frequency to the combined score of daytime frequencies and the score of nocturia was better in the Naf group (improved from 7.0 to 4.4, $P = 0.0017$) than in the Tam group (from 6.8 to 4.9, $P = 0.08$) ($P < 0.05$). At 6–8 weeks, the effects of the two drugs on lower urinary tract symptoms were comparable.

Conclusions: Naf demonstrated a significant early response to improve storage symptoms at 2 weeks, including daytime frequency and nocturia, compared with Tam.

Key words: alpha-receptor blocker, benign prostatic hypertrophy (BPH), International Prostatic Symptom Score (IPSS), lower urinary tract symptom (LUTS), storage symptom.

Introduction

Alpha-1-adrenoceptor antagonists have been the most frequently used initial treatment in elderly men with lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH). Three subtypes of the alpha-1-adrenoceptor (alpha-1-A, alpha-1-B, alpha-1-D) have been identified, and various types of alpha-1-adrenoceptor antagonists have become available worldwide. However, the comparative characteristics in both clinical efficacy and adverse effects of these agents are still controversial.[1–4] As reported, there are a greater number of elderly men with LUTS/BPH suffering from storage symptoms than those suffering from voiding symptoms. In particular, nocturia is the most significant symptom out of all LUTS to bother patients. This was revealed by self-reported questionnaires evaluating patients' specific experiences with each of the LUTS.[5] Interestingly, some clinical studies have emphasized the characteristics of naftopidil (Naf) to improve nocturia in men with LUTS.[6,7] The potential mechanisms in the release of storage symptoms by alpha-1-adrenoceptor antagonists involve (i) reducing bladder overactivity by their possible release of bladder outlet obstruction and/or (ii) their direct blockade of the up-regulated alpha-1-D-adrenoceptor subtype in the detrusor muscle and/or spinal cord nervous system.[8–11] In order to clarify the possible different clinical efficacies between Naf and tamsulosin hydrochloride (Tam), we conducted a randomized comparative study and analyzed the time course changes in the International Prostatic Symptom Score (IPSS), Quality of Life (QOL), and outcomes in uroflowmetry, with special reference to the outcomes at the two time points of 2 weeks and 6–8 weeks in the patients who had a two times or greater score of nocturia.

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Methods

The present study was conducted as a prospective, randomized controlled trial, and 13 urologists at 11 investigational sites participated in this multicentre trial. The procedures were permitted by the Ethical Committees for Clinical Research on Human Subjects at the relevant institutions. Men aged over 50 years with clinical symptoms of BPH were eligible for the study provided that they met the requirements described below.

The subjects were patients aged 50 years or above who, primarily due to LUTS, consulted the outpatient clinics of the Department of Urology, Kyoto Prefectural University of Medicine and the facilities of the Clinical Research Group on Urination Disorders at related hospitals between June 2004 and July 2007, who were diagnosed to have BPH and fulfilled the following criteria: (1) number of nocturia ≥2; (2) IPSS score ≥8; (3) QOL index ≥3; (4) residual urine volume <50 mL (evaluated by ultrasound estimation); (5) maximum voiding flow rate <15 mL/s (preferably with a urination volume ≥150 mL); and (6) prostate volume <50 mL (evaluated by transrectal ultrasound). Exclusions were made according to the following criteria: patients (1) who had prostate cancer, acute prostatitis, or narrowing of the urinary tract; (2) who received prostate surgery, balloon dilation, urinary tract stenting, hyperthermia, or pelvic radiation before the beginning of the study; (3) who were catheterized or were performing intermittent

Table 1 Patient characteristics at baseline

Variable	Naftopidil	*n*	Tamsulosin	*n*	*P*
Mean (SD):					
Age, years	69.6 (6.8)	31	68.8 (8.2)	28	$P = 0.66^{*1}$
Prostate volume, mL	24.4 (6.9)	31	26.7 (7.9)	28	$P = 0.20^{*1}$
TZ volume, mL	7.8 (4.5)		9.6 (4.2)		$P = 0.50^{*1}$
IPSS					
Daytime frequency	3.5 (1.6)	31	3.5 (1.6)	28	$P = 0.96^{*2}$
Urgency	2.2 (1.8)	31	2.4 (1.9)	28	$P = 0.73^{*2}$
Nocturia	3.5 (1.0)	31	3.4 (1.1)	28	$P = 0.48^{*2}$
Incomplete emptying	2.1 (1.8)	31	2.4 (2.0)	28	$P = 0.62^{*2}$
Intermittency	1.5 (1.7)	31	2.3 (2.1)	28	$P = 0.19^{*2}$
Slow stream	2.8 (1.9)	31	3.2 (1.8)	28	$P = 0.45^{*2}$
Straining	1.5 (1.7)	31	1.9 (1.8)	28	$P = 0.41^{*2}$
Total IPSS	17.2 (6.4)	31	18.9 (6.6)	28	$P = 0.28^{*2}$
Frequency					
Daytime frequency nocturia	7.0 (2.0)	31	6.8 (2.4)	28	$P = 0.84^{*2}$
Storage symptom					
Daytime frequency nocturia					
Urgency	9.3 (2.9)	31	9.3 (3.2)	28	$P = 0.95^{*2}$
Voiding symptom	5.8 (4.3)	31	7.3 (4.4)	28	$P = 0.18^{*2}$
QOL	4.7 (1.0)	31	4.8 (1.2)	27	$P = 0.79^{*2}$
Mean (SD):					
Uroflowmetry					
Qmax, mL/s	9.9 (5.5)	24	9.6 (4.8)	16	$P = 0.80^{*1}$
Qave, mL/s	4.8 (2.7)	24	3.6 (1.5)	16	$P = 0.12^{*1}$
PVR, mL	19.3 (26.1)	25	19.6 (20.2)	17	$P = 0.96^{*1}$

*1unpaired Student's *t*-test. *2Mann–Whitney *U*-test. IPSS, International Prostatic Symptom Score; PVR, postvoid residual urine volume; Qave, average urinary flow rate; Qmax, maximum urinary flow rate; QOL, quality of life; SD, standard deviation; TZ, transition zone.

self-catheterization; (4) who had marked night-time polyuria; (5) who had active urinary tract infection (urinary white blood cell count ≥5/hpf); (6) who were suspected to have neurogenic bladder or other neurological disorders; (7) who had severe ischemic heart disease, cerebrovascular disorders, liver dysfunction, or kidney dysfunction; (8) who had hypotension (systolic pressure ≤100 mmHg and diastolic pressure ≤60 mmHg), orthostatic hypotension, or severe hypertension; (9) who had developed hypersensitivity to Naf or Tam; (10) who had been administered a hormonal drug for prostatic hyperplasia within 1 month prior to the beginning of this study; (11) who had been administered a drug that might affect urination other than hormonal drugs for the treatment of prostatic hyperplasia within 2 weeks prior to the beginning of this study; (12) who were judged by the attending physicians to be inappropriate as subjects.

After consent had been obtained, the subjects were randomized into two groups: one for patients whose birthday was an odd number, for administration of 50 mg Naf; and the other for patients whose birthday was an even number, for administration of 0.2 mg Tam. The drugs were administered once a day for 6–8 weeks. The concomitant use of drugs that affect the urination function was forbidden.

The therapeutic effect and safety were evaluated 2 weeks and 6–8 weeks after the beginning of the treatments and at the end of observation. For statistical analyses, the data were expressed as the mean ± standard deviation. The Wilcoxon signed test, the Mann–Whitney *U*-test, the paired *t*-test, the unpaired *t*-test, one-way ANOVA, and a multiple comparison procedure (Scheffe's paired comparison) were used according to the nature of the data at a significance level of 5%.

At the beginning of administration, 2 weeks after the beginning of administration, and at the end of observation, the total IPSS and QOL score and the scores of individual IPSS items, storage symptoms, and voiding symptoms were evaluated. Combined variables to represent storage or voiding symptoms were also evaluated as the combined score of two or three items among storage or voiding symptoms.

At the beginning of administration and at the end of observation, the urination volume, maximum flow rate (Qmax), average flow rate (Qave), residual urine volume, and urination time were evaluated. In this study, evaluation of uroflowmetry was made in patients who voided 100 mL or more.

Results

Out of the 81 registered patients, data for 59 patients (59/81, 73%) consisting of 31 in the Naf group and 28 in the Tam group were analyzed. The reasons preventing registered patients from being analyzed were having prostate volume >50 mL, administration of Naf twice a day, or missing data.

Table 1 shows the characteristics of the subjects. No difference was noted between the two groups with regard to age, severity of urination disorders, prostate volume, and transition volume. Also, no difference was noted between the two groups concerning signs and symptoms such as the IPSS score and maximum voiding flow rate at the beginning of the study.

The total IPSS score decreased significantly 2 weeks after the beginning of administration and at the end of observation in both groups (Table 2). In the Naf group, total IPSS significantly decreased from

Table 2 Changes in the various clinical variables (each symptom of lower urinary tract symptom, total International Prostatic Symptom Score, and Quality of Life score) at the baseline and after 2 weeks and 6–8 weeks of treatment with naftopidil (Naf) and tamsulosin (Tam)

Variable	Baseline				At 2 weeks					At 6–8 weeks					Kruskal Wallis test	
	Naftopidil	*n*	Tamsulosin	*n*	Naftopidil	*n*	Tamsulosin	*n*	Intergroup P	Naftopidil	*n*	Tamsulosin	*n*	Intergroup P	Naftopidil	Tamsulosin
Median (SD):																
IPSS																
Daytime frequency	3.5 (1.6)	31	3.5 (1.6)	28	2.2 (1.5)	22	2.2 (1.8)	25	$P = 0.15$	1.6 (1.3)	25	2.0 (1.8)	23	$P = 0.12$	$P = 0.0001$	$P = 0.0128$
Intragroup *P*	–		–		$P = 0.0303$		$P = 0.10$			$P = 0.0001$		$P = 0.0249$				
Urgency	2.2 (1.8)	31	2.4 (1.9)	28	1.4 (1.5)	22	1.7 (2.1)	25	$P = 0.79$	1.0 (1.2)	25	1.3 (1.7)	23	$P = 0.78$	$P = 0.0294$	$P = 0.06$
Intragroup *P*	–		–		$P = 0.27$					$P = 0.0342$						
Nocturia	3.5 (1.0)	31	3.4 (1.1)	28	2.2 (1.0)	22	2.7 (1.3)	25	$P = 0.11$	1.6 (0.7)	25	1.7 (1.0)	23	$P = 0.38$	$P = 0.0000$	$P = 0.0000$
Intragroup *P*	–		–		$P = 0.0004$		$P = 0.23$			$P = 0.0000$		$P = 0.0000$				
Incomplete emptying	2.1 (1.8)	31	2.4 (2.0)	28	1.4 (1.2)	22	1.5 (1.1)	25	$P = 0.34$	1.1 (1.2)	25	1.0 (1.1)	23	$P = 0.84$	$P = 0.07$	$P = 0.0414$
Intragroup *P*	–		–		$P = 0.46$		$P = 0.61$			$P = 0.08$		$P = 0.0414$				
Intermittency	1.5 (1.7)	31	2.3 (2.1)	28	0.6 (1.2)	22	1.0 (1.3)	25	$P = 0.07$	0.6 (1.1)	25	1.0 (1.5)	23	$P = 0.68$	$P = 0.06$	$P = 0.06$
Intragroup *P*	–		–		$P = 0.1066$		$P = 0.20$			$P = 0.17$		$P = 0.10$				
Slow stream	2.8 (1.9)	31	3.2 (1.8)	28	1.3 (1.4)	22	1.4 (1.4)	25	$P = 0.63$	1.4 (1.4)	25	1.4 (1.3)	23	$P = 0.69$	$P = 0.0020$	$P = 0.0005$
Intragroup *P*	–		–		$P = 0.0093$		$P = 0.0094$			$P = 0.0149$		$P = 0.0030$				
Straining	1.5 (1.7)	31	1.9 (1.8)	28	0.4 (0.6)	22	1.1 (1.6)	25	$P = 0.56$	0.6 (0.8)	25	0.7 (1.0)	23	$P = 0.99$	$P = 0.0225$	$P = 0.0478$
Intragroup *P*	–		–		$P = 0.0363$		$P = 0.31$			$P = 0.14$		$P = 0.06$				
Total IPSS score	17.2 (6.4)	31	18.9 (6.6)	28	9.5 (5.4)	22	11.5 (6.8)	25	$P = 0.43$	7.8 (5.1)	25	9.2 (6.6)	23	$P = 0.98$	$P = 0.0000$	$P = 0.0000$
Intragroup *P*	–		–		$P = 0.0005$		$P = 0.0109$			$P = 0.0000$		$P = 0.0001$				
Frequency																
Daytime frequency																
Nocturia	7.0 (2.0)	31	6.8 (2.4)	28	4.4 (2.3)	22	4.9 (2.6)	25	$P = 0.0489$	3.2 (1.8)	25	3.7 (2.5)	23	$P = 0.10$	$P = 0.0000$	$P = 0.0003$
Intragroup *P*	–		–		$P = 0.0017$		$P = 0.08$			$P = 0.0000$		$P = 0.0005$				
Storage symptom																
Daytime frequency																
Nocturia																
Urgency	9.3 (2.9)	31	9.3 (3.2)	28	5.8 (3.4)	22	6.5 (3.7)	25	$P = 0.13$	4.2 (2.4)	25	5.0 (3.9)	23	$P = 0.22$	$P = 0.0000$	$P = 0.0003$
Intragroup *P*	–		–		$P = 0.0032$		$P = 0.08$			$P = 0.0000$		$P = 0.0005$				
Voiding symptom	5.8 (4.3)	31	7.3 (4.4)	28	2.2 (2.5)	22	3.5 (3.1)	25	$P = 0.90$	2.5 (2.9)	25	3.1 (2.8)	23	$P = 0.76$	$P = 0.0004$	$P = 0.0007$
Intragroup *P*	–		–		$P = 0.0026$		$P = 0.0210$			$P = 0.0062$		$P = 0.0020$				
QOL	4.7 (1.0)	31	4.8 (1.2)	27	3.3 (1.5)	20	3.5 (1.0)	14	$P = 0.34$	2.5 (1.3)	16	2.8 (1.3)	22	$P = 0.50$	$P = 0.0000$	$P = 0.0000$
Intragroup *P*	–		–		$P = 0.0022$		$P = 0.0211$			$P = 0.0000$		$P = 0.0000$				

The intergroup comparison used the Mann–Whitney *U*-test. Comparison of each group of before and after treatment (intragroup) used the Kruskal–Wallis test and post-hoc test. There was no significant difference in the Kruskal–Wallis test. IPSS, International Prostatic Symptom Score; QOL, Quality of Life; SD, standard deviation.

Table 3 Changes in the various clinical variables (uroflowmetry) at the baseline and after 6–8 weeks of treatment with naftopidil and tamsulosin

Variable	Baseline				At 6–8 weeks				
	Naftopidil	*n*	Tamsulosin	*n*	Naftopidil	*n*	Tamsulosin	*n*	Intergroup *P*
Mean (SD):									
Uroflowmetry									
Qmax, mL/s	10.7 (5.5)	25	11.8 (4.4)	14	12.0 (3.2)	18	14.6 (4.5)	13	$P = 0.46$
Intragroup *P*	–		–		$P = 0.13$		$P = 0.32$		
Qave, mL/s	5.1 (2.7)	25	4.1 (1.7)	11	6.8 (2.6)	18	6.8 (3.6)	13	$P = 0.64$
Intragroup *P*	–		–		$P = 0.0203$		$P = 0.10$		
PVR, mL	19.3 (26.1)	30	19.6 (20.2)	25	3.4 (5.4)	23	16.1 (24.9)	16	$P = 0.29$
Intragroup *P*	–		–		$P = 0.0023$		$P = 0.89$		
Voided volume	197.3 (97.7)	25	220.7 (138.5)	15	210.3	19	234.4 (136.5)	14	$P = 0.70$
Intergroup *P*	–		–		$P = 0.95$		$P = 0.34$		
Rate of PVR/Capacity, %	9.2 (11.7)	29	1.7 (3.0)	25	12.4 (13.5)	22	6.2 (9.6)	16	$P = 0.98$
Intergroup *P*	–		–		$P = 0.0021$		$P = 0.28$		
Voiding time	44.6 (22.2)	25	53.6 (25.1)	11	33.6 (15.1)	18	44.8 (33.7)	13	$P = 0.36$
Intergroup *P*	–		–		$P = 0.0498$		$P = 0.97$		

The inter-group comparison by the Mann–Whitney *U*-test (before baseline vs after treatment). The comparison of each group between before and after treatment (intragroup) by the paired Student's *t*-test. PVR, postvoid residual urine volume; Qave, average urinary flow rate; Qmax, maximum urinary flow rate; SD, standard deviation.

17.2 ± 6.4 to 9.5 after 2 weeks ($P = 0.0005$) and 7.8 at the end of administration ($P < 0.0001$). In the Tam group, total IPSS significantly decreased from 18.9 to 11.5 after 2 weeks ($P = 0.011$) and 9.2 at the end of administration ($P < 0.0001$). The QOL index also improved in both groups similarly to the total IPSS score. However, no significant difference was noted in these scores between the two groups either after 2 weeks or at the end of administration (Table 2).

At 2 weeks in the Naf group, the score of daytime frequency significantly improved from 3.5 to 2.2 ($P = 0.03$), and the score of nocturia improved significantly from 3.5 to 2.2 ($P = 0.0004$). In the Tam group at 2 weeks, however, no significant improvement was noted in the score of daytime frequency (changed from 3.5 to 2.2, $P = 0.1$) or the score of nocturia (changed from 3.4 to 2.7, $P = 0.1$). At 2 weeks, the combined storage symptom score of urinary frequency, representing both the score of daytime frequency and the score of nocturia, was better in the Naf group (improved from 7.0 to 4.4, $P = 0.0017$) than in the Tam group (changed from 6.8 to 4.9, $P = 0.08$) ($P = 0.0489$). At 2 weeks, in analyzing the total storage symptom score to the sum of all the scores of daytime frequency, urgency, and nocturia, it improved from 9.3 to 5.8 ($P = 0.0032$) in the Naf group, while it was not statistically improved in the Tam group (changed from 9.3 to 6.5, $P = 0.08$). This intergroup analysis at 2 weeks suggested that Naf had better effects to improve storage symptoms in the acute phase (2 weeks) than Tam.

At the end of administration (6–8 weeks) in the Naf group, concerning the storage symptoms, the score of daytime frequency significantly improved from 3.5 to 1.6 ($P = 0.0001$), and the score of nocturia significantly improved from 3.5 to 1.6 ($P < 0.0001$). Concerning the voiding symptoms, the score of slow stream significantly improved from 2.8 to 1.4, ($P = 0.015$). In the Tam group at the end of administration (6–8 weeks), concerning the storage symptoms, the score of daytime frequency significantly improved from 3.5 to 2.0 ($P = 0.025$), and the score of nocturia significantly improved from 3.4 to 1.7 ($P < 0.0001$). Concerning the voiding symptoms, the score of slow stream significantly improved from 3.2 to 1.4 ($P = 0.003$). Notably, in the intergroup analysis at 6–8 weeks between Tam and Naf, the effects of the two drugs on LUTS were comparable (Table 2).

When the data of uroflowmetry were analyzed in patients who voided 100 mL or more in urine volume, 40 data (40/59, 68%) before administration and 33 data (34/59, 58%) at the end of the administration were able to be analyzed. No significant difference was noted between the Naf and Tam groups before administration (Table 3). In the Naf group, the mean voiding flow rate (mL/s) significantly increased from 5.1 to 6.8 ($P = 0.02$), and the residual urine volume (mL) significantly decreased from 19.3 to 3.4 ($P = 0.0023$) at the end of administration (6–8 weeks).

Discussion

This study had fundamental limitations because of the small number of patients ($n = 59$), the multi-institutional nature of the study, and the modest percentage (73%) of the available analysis rate. However, our data successfully duplicated the previously reported comparative findings for Tam and Naf to be equally effective in improvement of both storage and voiding symptoms at 6–8 weeks administration, which was described in the greater numbered randomized controlled study.[12] In this study, especially, to compare the early effects of Naf and Tam, the effects at 2 weeks of administration demonstrated significant difference in improvement of the storage symptom to be characterized by combined scores of daytime frequency and nocturia. This finding might allow us to discuss the possible different clinical efficacy between Naf and Tam.

Recent studies on alpha-1 receptors have shown the presence of three subtypes: alpha-1A, alpha-1B, and alpha-1D.[13,14] There have been reports that alpha-1A and alpha-1D receptors are expressed at high levels in hyperplastic human prostates[15] and that their distribution shows wide individual variation.[16] Recent studies provided further interesting evidence on the difference in the receptors not only in the prostate but also in the bladder or nervous system. Details of the pathology of overactive bladder still remain controversial, but an involvement of various neuromuscular receptors present in the detrusor muscles and bladder epithelium has been suggested.[17] Interestingly, alpha-1D receptors in the bladder, urinary tract, and central nervous

system have been speculated to be involved in overactive bladder.[18–20] This speculation has been derived from reports that the alpha-1D subtype is dominant in the detrusor muscles of the human bladder and parasympathetic ganglia in the human sacral spinal cord[21,22] and the recent reports that alpha-1D receptors are present in bladder epithelial cells and are involved in the control of contraction and relaxation of bladder smooth muscles by releasing adenosine tri-phosphate (ATP) and transmitting excitation to afferent nerve terminals.[22,23]

Naf and Tam are widely used as the first choices for the treatment of LUTS associated with BPH.[24,25] Although both Tam and Naf are categorized as alpha-1A/alpha-1D antagonists, Tam seems to have a relatively higher affinity and selectivity to the alpha-1A receptor subtype while Naf seems to have a relatively higher affinity to the alpha-1D receptor subtype. Although the difference in affinity to the alpha-1D receptor between these two drugs seems not so much *in vitro*, there is still controversy over the possible differences in their therapeutic effects. There have been some reports that the effectiveness of the two drugs on voiding symptoms as a whole was comparable at 4 or more weeks' administration.[12] Others report that Naf showed better effects on storage symptoms especially on increased daytime frequency at 8 weeks or on nocturia at 4 weeks administration, although Tam was superior to Naf on intermittency.[7,25] To our knowledge, there has been no report on evaluation of the early effects such as at 2 weeks after the beginning of administration. In the present study, Naf showed a greater improvement in the score of the entire urinary frequency, representing combined scores of daytime frequency and nocturia, than Tam at 2 weeks after the beginning of the treatments, although we found no statistical difference between the two groups in individual score in the items of daytime frequency and nocturia.

The report by Sugaya *et al.*[11] on the inhibitory effect on rhythmic contraction of the bladder in rats and the one by Yokoyama *et al.*[20] on the increase in bladder capacity associated with the inhibition of C fibers in rats with cerebrovascular disorders suggest that the early improvements in storage symptoms due to Naf administration were due to its action on the nervous systems involved in the bladder function. That is, the improvements in storage symptoms observed early after the beginning of Naf administration may have been caused primarily by inhibition of stimulation of afferent fibers due to its action on alpha-1D receptors. Moreover, our results suggest that overactive bladder, the etiology of which remains difficult to determine, may be caused by activities of the central nervous system in which alpha-1D receptors are involved.

The reason for the early response of Naf to improve scores at 2 weeks combining daytime frequency and nocturia in this study is hardly conclusively explained. However, the results might allow us to discuss the speculation that because of the distribution of alpha-1D receptors, Naf may have a stronger action on the central nervous system and exert a greater inhibitory effect on afferent stimulation in the bladder, with more rapid action on storage symptoms because of its effect on nerves with alpha-1D receptors. These characteristics explain the early improvements in storage symptoms of frequency at 2 weeks. However, as Tam relieved functional obstruction of the lower urinary tract by inducing relaxation of smooth muscle, it may have mitigated storage symptoms, causing improvements in the number of urinations, and the difference in the effects of the two drugs on voiding and storage symptoms are considered to have disappeared after 6 or more weeks. In addition, after 6–8 weeks, Naf characteristically reduced the urgency while Tam characteristically alleviated the feeling of incomplete emptying, probably reflecting differences in their actions. Also, alpha-1D receptors have been found to be involved in the storage of urine in the bladder as was suggested by the report by Chen *et al.* that the bladder capacity and urine volume per urination were significantly higher in alpha-1D knock-out mice than wild mice.[25] Thus, this report suggests that with relatively greater affinity to alpha-1D receptors, Naf can affect the storage function. This does not contradict the clinical reports that the improvements in storage symptoms by Naf administration are caused by an improvement in bladder compliance.[7]

Conclusions

This study showed that with a relatively higher affinity to the alpha-1D receptor subtype, Naf characteristically causes early improvements in increased frequency of urination compared with Tam, with a relatively higher affinity to alpha-1A. This information is considered to contribute to the selection of drugs for the treatment of BPH patients with storage symptoms who must void two or more times during the nighttime.

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Anti-Gout Agent Allopurinol Exerts Cytotoxicity to Human Hormone-Refractory Prostate Cancer Cells in Combination with Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand

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Abstract

Allopurinol has been used for the treatment of gout and conditions associated with hyperuricemia for several decades. We explored the potential of allopurinol on cancer treatment. Allopurinol did not expose cytotoxicity as a single treatment in human hormone refractory prostate cancer cell lines, PC-3 and DU145. However, allopurinol drastically induced apoptosis of PC-3 and DU145 in combination with tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), which is a promising candidate for anticancer agent but its efficacy is limited by the existence of resistant cancer cells. We examined the underlying mechanism by which allopurinol overcomes the resistance of prostate cancer cells to TRAIL. Allopurinol up-regulated the expression of a proapoptotic TRAIL receptor, death receptor 5 (DR5). Allopurinol increased DR5 protein, mRNA, and promoter activity. Using DR5 small interfering RNA (siRNA), we showed that allopurinol-mediated DR5 up-regulation contributed to the enhancement of TRAIL effect by allopurinol. Furthermore, we examined the mechanism of allopurinol-mediated DR5 up-regulation. DR5 promoter activity induced by allopurinol was diminished by a mutation of a CAAT/enhancer binding protein homologous protein (CHOP)-binding site. In addition, allopurinol also increased CHOP expression, suggesting that allopurinol induced DR5 expression via CHOP. Allopurinol possesses the activity of a xanthine oxidase (XO) inhibitor. We used XO siRNA instead of allopurinol. XO siRNA also up-regulated DR5 and CHOP expression and sensitized the prostate cancer cells to TRAIL-induced apoptosis. Here, we show the novel potential of allopurinol in cancer treatment and indicate that the combination of allopurinol with TRAIL is effective strategy to expand the TRAIL-mediated cancer therapy. (Mol Cancer Res 2008;6(12):1852–60)

Introduction

Prostate cancer is the most common malignancy and the second leading cause of male cancer death in the United States. The American Cancer Society estimated that, during 2006, about 234,460 new cases of prostate cancer would be diagnosed in the United States and 27,350 men would die of metastatic disease (1). Although androgen ablation is effective in treating prostate cancer, most patients become resistant to hormonal manipulation (2, 3); therefore, new treatment strategies are needed for this disease.

Allopurinol has been a cornerstone of the clinical management of gout and conditions associated with hyperuricemia and has been used worldwide since 1966 (4). Allopurinol acts as a xanthine oxidase (XO) inhibitor and recent data indicate that XO also plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases, and chronic heart failure (5-7). Allopurinol has shown a beneficial effect in the treatment of these conditions both in experimental animal models and in human clinical trials (5-7). Thus, allopurinol has many clinical benefits; however, it has not been applied to cancer treatment.

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL/Apo2L) induces apoptosis selectively in cancer cells *in vitro* and *in vivo* and has little or no toxicity to normal cells (8-12). Recombinant TRAIL and agonistic TRAIL receptor antibodies are promising for cancer treatment and going on phase I/II clinical studies (13, 14). TRAIL is a cytokine that is closely related to TNF-α and Fas ligand, members of the TNF family (15). Death receptor 5 (DR5; also called TRAIL-R2) is a receptor for TRAIL. TRAIL induces apoptosis by binding to DR5, causing the formation of a death-inducing signaling complex with binding of caspase-8 (16-21). Autoactivated caspase-8 can directly evoke the cleavage of downstream effector caspases (22, 23). However, some tumor types exhibit resistance to TRAIL (24) and it is important to overcome this resistance.

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In the present study, we searched a potential of allopurinol in hormone-resistant prostate cancer treatment and found that allopurinol dramatically causes apoptosis of prostate cancer cells in combination with TRAIL.

Results

Allopurinol Exerts Cytotoxicity of Hormone-Resistant Human Prostate Cancer Cells in Combination with TRAIL

First, we investigated the cytotoxic effect of allopurinol on hormone-resistant human prostate cancer cells, PC-3 and DU145, as a single agent. As shown in Fig. 1A, allopurinol did not have cytotoxic effect in both PC-3 and DU145 cells. Both cells were also resistant to TRAIL-induced apoptosis (Fig. 1B). Interestingly, allopurinol markedly induced cytotoxic effect on PC-3 and DU145 cells when combined with TRAIL. To elucidate that the sub G_1 population caused by the combination of allopurinol and TRAIL is caspase-dependent apoptosis, we used caspase inhibitors. The pan-caspase inhibitor zVAD-fmk efficiently blocked the sub G_1 induced by combined treatment with allopurinol and TRAIL (Fig. 1C). These results indicate that the cytotoxic effect mediated by

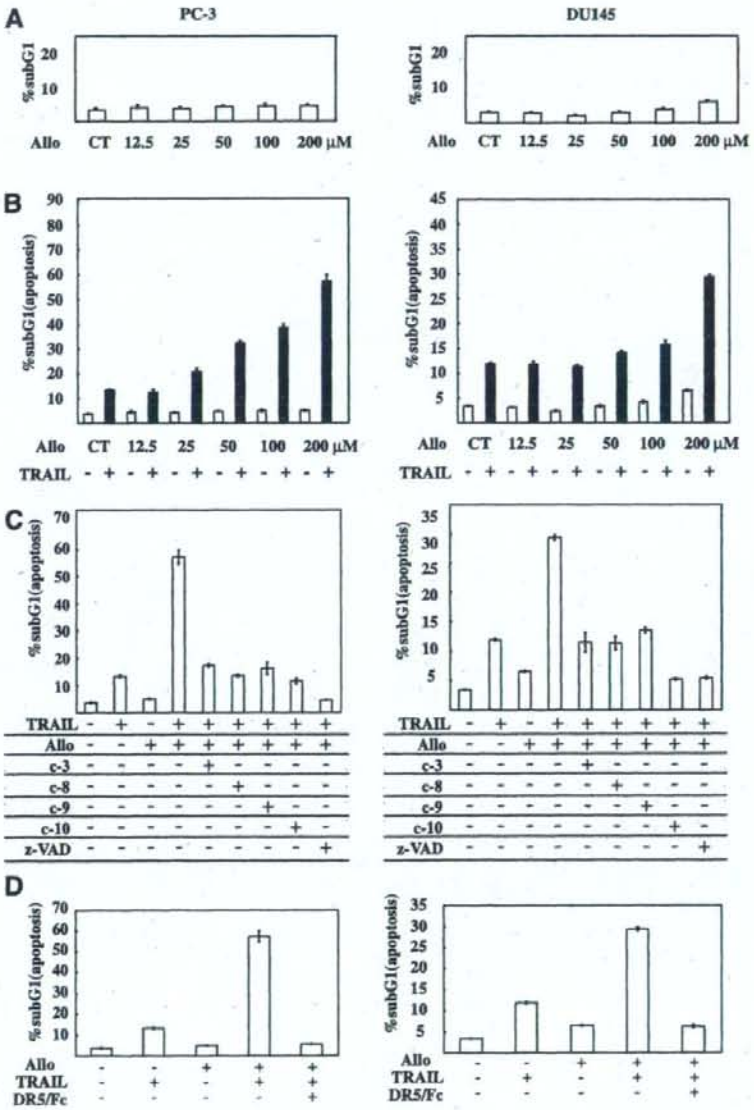

FIGURE 1. Sensitization to TRAIL-induced apoptosis by allopurinol in PC-3 and DU145 cells. **A.** Allopurinol treatment alone does not induce apoptosis. PC-3 and DU145 cells were treated with allopurinol at the indicated concentrations for 24 h. CT, treated with DMSO (control). **B.** Allopurinol potentiates TRAIL-induced apoptosis. PC-3 and DU145 cells were treated with allopurinol and/or TRAIL (PC-3, 5 ng/mL; DU145, 50 ng/mL) at the indicated concentrations for 24 h. **C.** Caspase inhibitors reduce the sensitization to TRAIL-induced apoptosis by allopurinol in PC-3 and DU145 cells. Cells were treated with allopurinol (200 μmol/L), TRAIL (PC-3, 5 ng/mL; DU145, 50 ng/mL), and/or various caspase inhibitors (20 μmol/L). c-3, zDEAD-fmk caspase-3 inhibitor; c-8, zIETD-fmk caspase-8 inhibitor; c-9, zLEHD-fmk caspase-9 inhibitor; c-10, zAEVD-fmk caspase-10 inhibitor; zVAD, zVAD-fmk pan-caspase inhibitor. **D.** DR5/Fc chimeric protein blocks the enhancement of TRAIL-induced apoptosis by allopurinol. Cells were treated with allopurinol (200 μmol/L), TRAIL (PC-3, 5 ng/mL; DU145, 50 ng/mL), and/or DR5/Fc chimera (1 μg/mL) for 24 h. In **A** to **D**, apoptosis was determined by FACS analysis of the DNA fragmentation of propidium iodide–stained nuclei as described in Materials and Methods. Columns, mean of triplicate experiments; bars, SD.

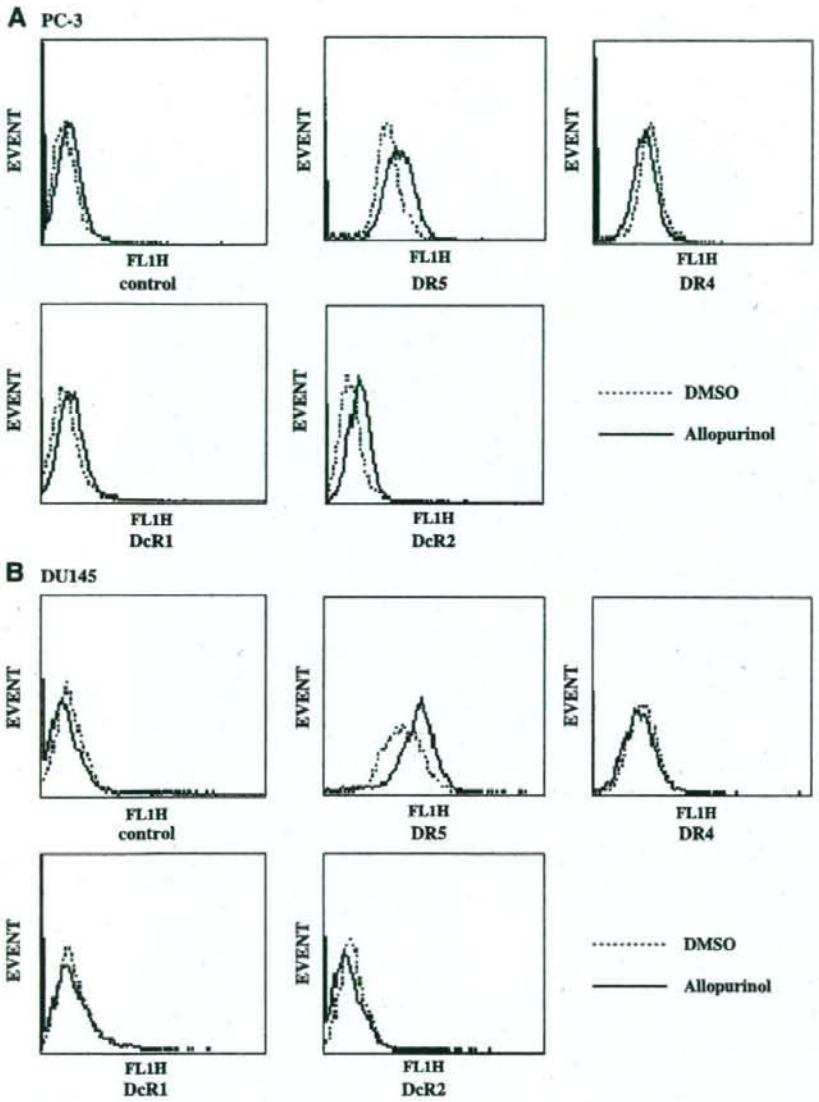

FIGURE 2. Allopurinol increases cell surface DR5 expression in both PC-3 and DU145 cells. **A.** PC-3. **B.** DU145. Cells were treated with 0.1% DMSO or 200 μmol/L allopurinol for 24 h. Subsequently, cells were stained with isotype control IgG and monoclonal antibodies were generated against the extracellular domain of TRAIL receptors DR5, DR4, DcR1, and DcR2. Data were analyzed by flow cytometry. Dotted line histogram, DMSO; solid line histogram, allopurinol.

allopurinol and TRAIL stands for caspase-dependent apoptosis. Moreover, caspase-3–like, caspase-8–like, caspase-9–like, and caspase-10–like inhibitors (25) also interrupted the apoptosis induced by combined treatment. To elucidate whether apoptosis induced by allopurinol and TRAIL occurred via a specific interaction between TRAIL and its receptors, we used a recombinant human DR5/Fc chimeric protein, which has a dominant-negative effect by competing with endogenous TRAIL receptors. As shown in Fig. 1D, the allopurinol-mediated enhancement of TRAIL-induced apoptosis was markedly blocked by DR5/Fc chimera, indicating that allopurinol sensitizes prostate cancer cells to TRAIL-induced apoptosis through specific interactions of TRAIL with its receptors.

Allopurinol Increases a TRAIL Receptor, DR5, Expression in Both PC-3 and DU145 Cells

To elucidate how allopurinol sensitizes prostate cancer cells to TRAIL action, we examined cell surface TRAIL receptor expression by flow cytometry. As shown in Fig. 2, allopurinol increased cell surface DR5 expression in both PC-3 and DU145 cells. In contrast, DR4 and decoy receptor 1 (DcR1) remained unchanged in both cells, although DcR2 was slightly increased

in only PC-3 cells. These results indicate that allopurinol up-regulates DR5 among TRAIL receptors. We carried out Western blotting to investigate the induction of DR5 by allopurinol at a total protein level. Allopurinol increased DR5 protein in both PC-3 and DU145 cells (Fig. 3A). Moreover, DR5 mRNA was also increased by allopurinol treatment (Fig. 3B). To investigate the further mechanism underlying DR5 up-regulation by allopurinol, we next examined the effect of allopurinol on DR5 promoter activity. We carried out a luciferase assay using reporter plasmids containing the DR5 promoter. Allopurinol significantly enhanced DR5 promoter activity in both PC-3 and DU145 cells (Fig. 3C). These results indicate that allopurinol regulates DR5 expression through transcription.

Up-Regulation of DR5 by Allopurinol Contributes to the Enhancement of TRAIL-Induced Apoptosis

Next, we tested whether up-regulation of DR5 expression by allopurinol has an effect on TRAIL-induced apoptosis. The expression of DR5 protein was efficiently reduced by transiently transfected DR5 small interfering RNA (siRNA; Fig. 4A). This reduction of DR5 expression significantly attenuated the apoptotic response to combined treatment with allopurinol and TRAIL (Fig. 4B). These results suggest that the up-regulation of DR5 expression accounts, at least in part, for the synergistic enhancement of TRAIL-induced apoptosis by allopurinol.

Identification of Allopurinol-Responsive Elements in the DR5 Promoter

As shown in Fig. 3C, allopurinol enhanced DR5 promoter activity in both PC-3 and DU145 cells. Using a series of 5′-deletion mutants, we investigated allopurinol-responsive elements on the DR5 promoter. As shown in Fig. 5A, luciferase activity from pDR5/−347 as well as pDR5PF (−2.5 kbp) was increased by allopurinol. On the other hand, pDR5/−252 showed a lack of response following allopurinol treatment. These results indicate that the major allopurinol response elements are located between −347 and −253 in the DR5 promoter. This region contains a potential CAAT/enhancer binding protein homologous protein (CHOP)-binding site. To determine whether the site is responsible for transactivation of the DR5 promoter by allopurinol, we carried out a luciferase assay with pDR5/mtCHOP containing a point mutation in the CHOP-binding site. The mutation abolished activation of the DR5 promoter by allopurinol (Fig. 5B). These results suggest that CHOP is associated with DR5 up-regulation by allopurinol.

Allopurinol Increases CHOP Protein, mRNA, and Promoter Activity via Endoplasmic Reticulum Stress Element–Independent Pathway

Allopurinol treatment induced CHOP protein in a dose-dependent manner in both PC-3 and DU145 cells (Fig. 6A). Allopurinol also up-regulated CHOP mRNA (Fig. 6B). To elucidate the mechanism of CHOP up-regulation by allopurinol, we carried out a luciferase assay using reporter plasmids containing the CHOP promoter. Allopurinol increased the promoter activity of CHOP3K, a luciferase reporter plasmid containing a ~3 kbp fragment of the CHOP promoter region (Fig. 7A). Previous reports have shown that endoplasmic reticulum stress element (ERSE) on the CHOP gene promoter is activated by ER stress triggered by tunicamycin (26). Therefore, to determine whether transactivation of CHOP promoter by allopurinol is caused by ER stress, we carried out a luciferase assay with pCHOP/mtERSE that was developed and described previously (27). The mutation of ERSE abolished the activation of the CHOP promoter by tunicamycin, although

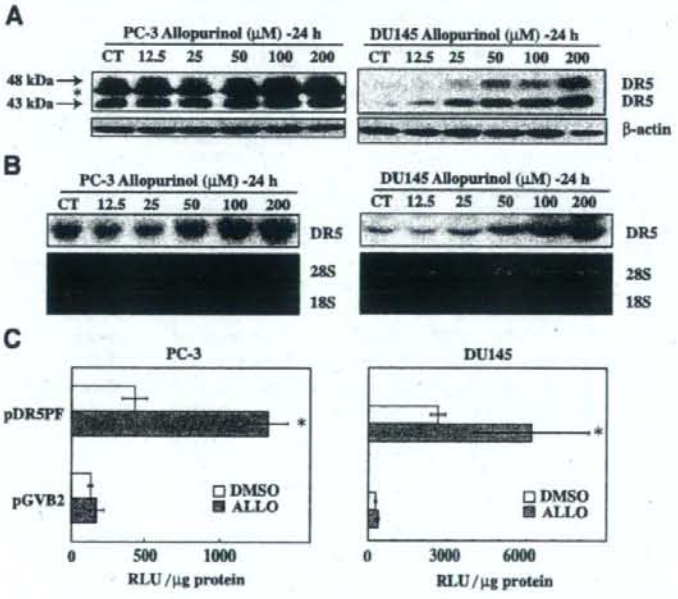

FIGURE 3. Allopurinol up-regulates DR5 expression in PC-3 and DU145 cells. **A.** Allopurinol up-regulates DR5 protein expression. PC-3 and DU145 cells were treated with allopurinol at the indicated concentrations for 24 h. CT, treated with DMSO (control). Arrows indicate DR5 proteins. Asterisk indicates nonspecific band. The 48- and 43-kDa bands correspond to long and short DR5 isoforms, respectively. **B.** Allopurinol up-regulates DR5 mRNA expression. PC-3 and DU145 cells were treated with allopurinol at the indicated concentrations for 24 h. Northern blotting was done as described in Materials and Methods. Ethidium bromide–stained 28S and 18S rRNA are shown as controls. CT, treated with DMSO (control). **C.** Allopurinol (*ALLO*) enhanced DR5 promoter activity. Luciferase assay was carried out with PC-3 and DU145 cells treated with 200 μmol/L allopurinol for 24 h after transfection of a luciferase plasmid containing DR5 promoter (pDR5PF). pGVB2 is a vacant control plasmid. Columns, mean of triplicate experiments; bars, SD. *, $P < 0.05$. RLU, relative luciferase units.

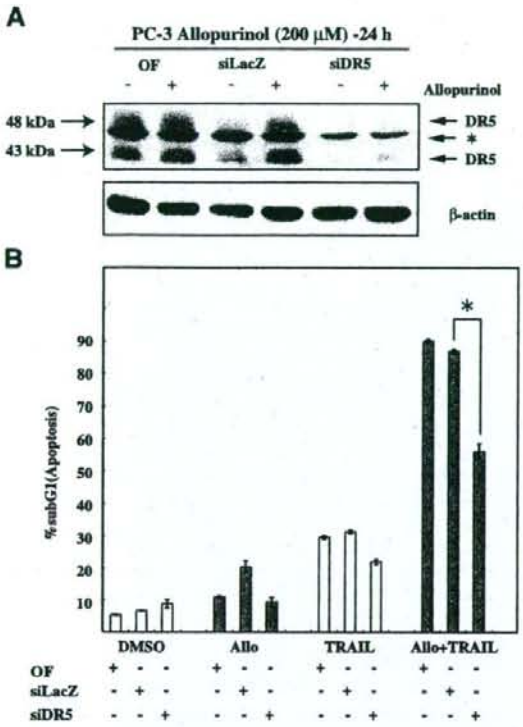

FIGURE 4. Down-regulation of DR5 reduces allopurinol-mediated TRAIL-induced apoptosis in PC-3 cells. **A.** Reduction of DR5 protein by DR5 siRNA. PC-3 cells were treated with DR5 siRNA, LacZ siRNA, or transfection reagent (Oligofectamine) alone. Twenty-four hours after transfection, cells were treated with allopurinol (200 μmol/L) for 24 h. Western blotting was done as described in Materials and Methods. β-actin was used to ensure equal gel loading. Arrows indicate DR5 proteins. Asterisk indicates nonspecific band. The 48- and 43-kDa bands correspond to long and short DR5 isoforms, respectively. **B.** Inhibition of DR5 expression reduces the sensitization to TRAIL-induced apoptosis by allopurinol. PC-3 cells were treated with DR5 siRNA, LacZ siRNA, or transfection reagent alone. Twenty-four hours after transfection, cells were treated with allopurinol (200 μmol/L) and/or TRAIL (5 ng/mL) for 24 h. Apoptosis was determined by FACS analysis of the DNA fragmentation of propidium iodide–stained nuclei as described in Materials and Methods. Data, mean of triplicate experiments; bars, SD. *, $P < 0.05$.

tunicamycin increased the activity of pCHOP/−150 in PC-3 and DU145 cells (Fig. 7B and C). However, both pCHOP/−150 and pCHOP/mtERSE showed a lack of response following allopurinol treatment in both cell lines (Fig. 7B and C). These results indicate that ER stress is not associated with CHOP up-regulation by allopurinol. Next, to identify the allopurinol-responsive element, we generated 5′-deletion mutants between −3026 and −151 and did the luciferase assay. As shown in Fig. 7D, the allopurinol-responsive element is located in a 36-bp region between −256 and −220 in the CHOP promoter. This allopurinol-responsive region contains an activator protein-1 (AP-1)–binding site and a heat shock factor (HSF)-binding site. Therefore, we introduced site-directed mutations into the sites to generate pCHOP/mtAP-1 and pCHOP/mtHSF from pCHOP/−256. Allopurinol at 200 μmol/L enhanced the promoter activity from pCHOP/−256 to 2.8-fold. The induction was reduced to 1.8- and 2.1-fold by the mutations at AP-1 and HSF site, respectively. These results suggest that AP-1 and HSF sites may be partly responsible for the activation of CHOP promoter by allopurinol, but other sites may also exist in the 36-bp region.

Xanthine Oxidase–Specific siRNA Up-Regulates DR5 Expression and Enhances TRAIL-Induced Apoptosis in PC-3 Cells

To examine the involvement of XO inhibition in the allopurinol effects, we investigated the effect of XO-specific siRNA on DR5 expression. As shown in Fig. 8A, transfection of XO siRNA suppressed XO expression compared with cells transfected with control siRNA. Figure 8A shows the effect of silencing XO on DR5 and CHOP protein expression. XO siRNA up-regulated DR5 and CHOP expression in PC-3 cells. Moreover, combined treatment with XO siRNA and TRAIL markedly induced apoptosis (Fig. 8B). These results indicate that inhibition of XO plays an essential role in the up-regulation of DR5 through CHOP and in enhancing TRAIL-induced apoptosis by allopurinol.

Discussion

To date, anticancer activity of allopurinol has not been reported. In this report, we show for the first time that allopurinol drastically induces apoptosis in human hormone-refractory prostate cancer cells when combined with TRAIL. Our present data indicate the novel potential of allopurinol as an anticancer agent.

Moreover, as a novel molecular action of allopurinol, we showed that allopurinol up-regulated DR5 and CHOP expression through a transcription. The observation is very surprising because allopurinol functions as a gene expression regulator. Previously, we showed that tunicamycin, an ER stress inducer, up-regulates DR5 and CHOP expression; however, we revealed here that allopurinol regulates the expression in a different manner from ER stress.

Using XO siRNA instead of allopurinol, we showed for the first time that XO knockdown enhances TRAIL-induced apoptosis with increased CHOP and DR5 expression. It has been reported that XO activity is high in brain tumor, lung cancer, colorectal cancer, and damaged tissue but not in normal tissues (28-30); therefore, allopurinol plus TRAIL might specifically induce apoptosis in cancer cells but not in normal cells.

Our study has important implications in cancer treatment. As mentioned above, allopurinol enables to overcome resistance to TRAIL and hormone in prostate cancer. Furthermore, more than a half of all malignant tumors possess an inactivating mutation in the *p53* gene and *p53* modulates the sensitivity against conventional anticancer agents (31, 32). Because PC-3 and DU145 cells harbored inactivated *p53*, combined treatment with allopurinol and TRAIL is also useful for *p53*-deficient tumor cells.

In conclusion, we have shown that allopurinol cooperates with TRAIL and induces apoptosis in prostate cancer cells via DR5 up-regulation caused by the inhibition of XO. These results suggest that the combined treatment of allopurinol with TRAIL may be promising for the treatment of hormone-refractory prostate cancer. At present, effects of anticancer

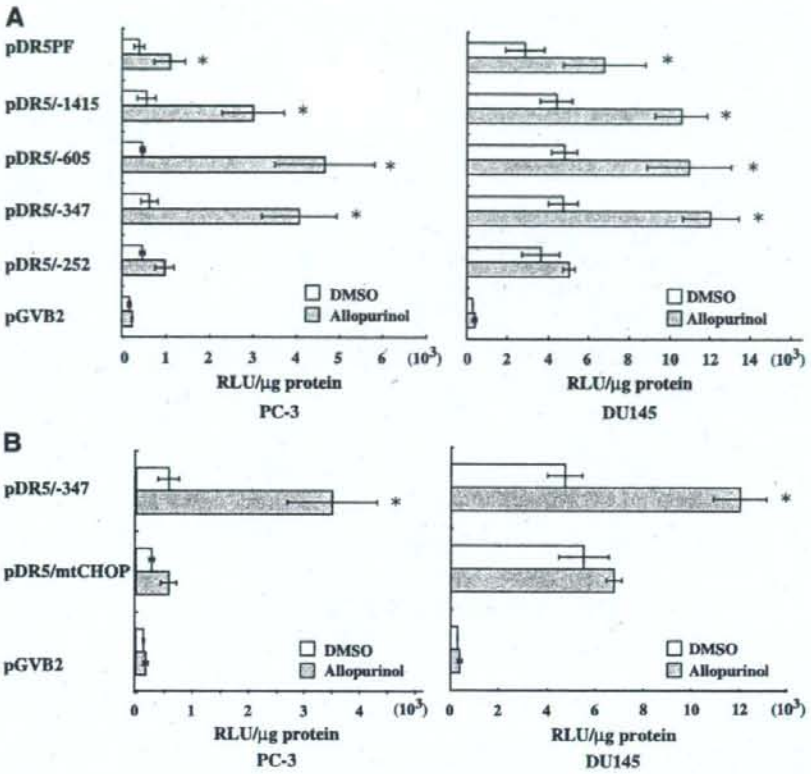

FIGURE 5. Identification of allopurinol-responsive elements in the DR5 promoter. **A** and **B.** Luciferase activity in transiently transfected PC-3 and DU145 cells treated with or without allopurinol (200 μmol/L) for 24 h. Luciferase assays were done as described in Materials and Methods. Data, mean of triplicate experiments; bars, SD. *, $P < 0.05$.

agent in a single use are limited. Our present data raise a possibility that the combined use of agents that have clinical benefits in other disease expands the ability of each agent and becomes useful for cancer treatment.

Materials and Methods

Reagents

Allopurinol, tunicamycin, and soluble recombinant human TRAIL/Apo2L were purchased from Sigma and PeproTech, respectively. Recombinant human DR5 (TRAIL-R2)/Fc chimera and the caspase inhibitors zVAD-fmk, zDEVD-fmk, zIETD-fmk, zLEHD-fmk, and zAEVD-fmk were purchased from R&D Systems.

Cell Culture

Human prostate cancer cell lines, PC-3 and DU145, were maintained in RPMI 1640 with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2.

Western Blot Analysis

Western blot analysis was done as previously described (33) using rabbit polyclonal anti-DR5 antibody (1:250; Prosci);

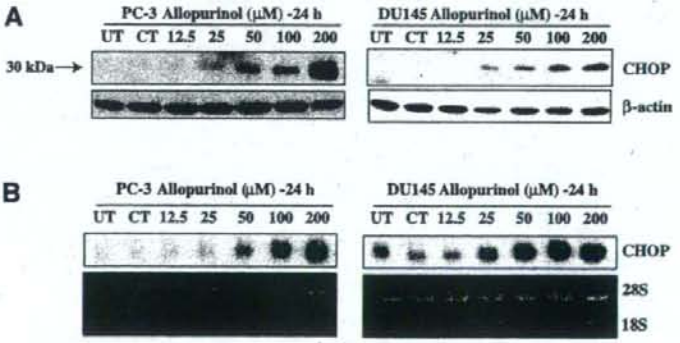

FIGURE 6. CHOP protein and mRNA are increased by allopurinol treatment. **A.** Allopurinol up-regulates CHOP protein expression in PC-3 and DU145 cells. Cells were treated with allopurinol at the indicated concentrations for 24 h. Western blotting was done as described in Materials and Methods. β-actin was used to ensure equal gel loading. **B.** Allopurinol up-regulates CHOP mRNA. PC-3 and DU145 cells were treated with the indicated concentrations of allopurinol for 24 h. Northern blotting was done as described in Materials and Methods. 28S and 18S rRNA are loading controls. UT, untreated; CT, treated with DMSO (control).

anti-CHOP (1:200) and anti-XO (1:100) antibodies (Santa Cruz Biotechnology) and mouse monoclonal anti-β-actin antibody (1:1,000) were used for detection (Sigma).

Northern Blot Analysis

Northern blot analysis was done as previously described using full-length DR5 or CHOP cDNA as a probe (34).

Plasmid Preparation

pDR5PF and deletion mutant plasmids containing DR5 promoter were previously described (35). CHOP3K and deletion mutant plasmids containing CHOP promoter were previously described (27). pCHOP/−256, pCHOP/−220, and pCHOP/−150 were generated by self-ligation following *Sac*I and *Sac*II digestion and Klenow fragment treatment. pCHOP/mtAP-1 and pCHOP/mtHSF were generated with a site-directed mutagenesis kit (Stratagene).

Transfection and Luciferase Assay

A series of DR5 and CHOP reporter plasmids and vacant vector plasmid (1.0 μg) were transfected into PC-3 and DU145 cells (1.5×10^5) using the DEAE-dextran method (CellPhect, GE Healthcare). After 24 h, the cells were treated with or without allopurinol for 24 h and then harvested. Levels of luciferase activity were normalized with protein concentrations. Luciferase assays were carried out in triplicate, and the experiments were repeated several times. Data were analyzed using Student's *t* test, and differences between DMSO and allopurinol treatment were considered significant when $P < 0.05$.

Determination of Apo2L/TRAIL Receptor Expression

As previously described (36), cells were harvested by short trypsinization, washed once with ice-cold PBS containing 1% bovine serum albumin, and resuspended in 100 μL PBS with 1% bovine serum albumin. Then, 5 μg of phycoerythrin-labeled

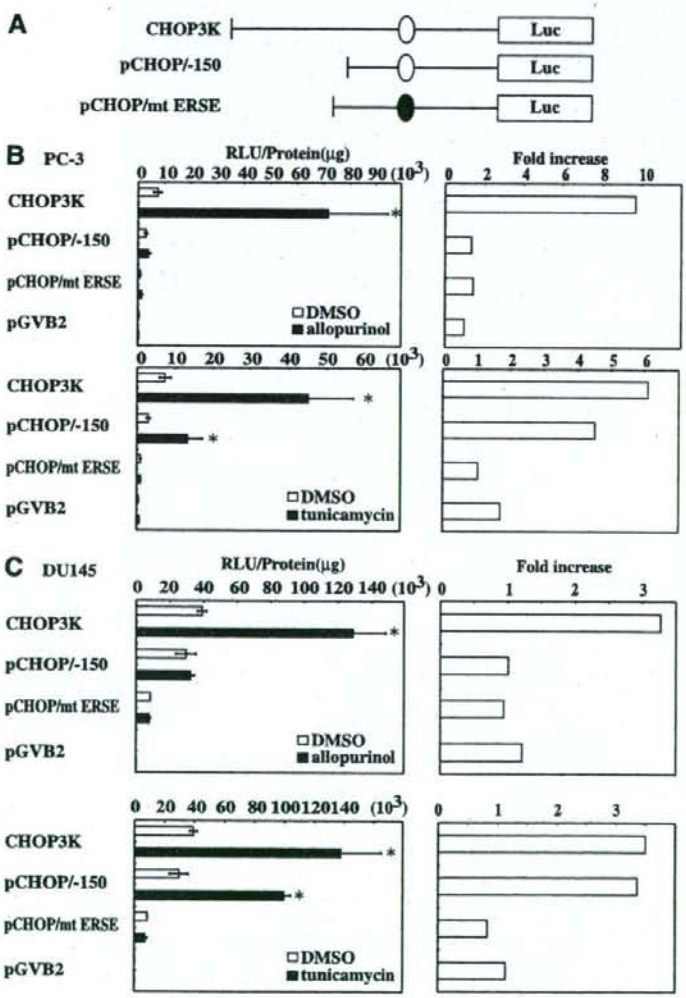

FIGURE 7. Allopurinol enhances CHOP promoter activity via ERSE-independent pathway. **A.** Structures of CHOP promoter-luciferase reporter plasmids. Circles indicate ERSE. Open circle, wild-type; closed circle, mutant. **B** and **C.** Allopurinol enhanced CHOP promoter activity in PC-3 and DU145 cells. Luciferase assay was carried out with PC-3 (**B**) and DU145 (**C**) cells treated with 200 μmol/L allopurinol, 1 μg/mL tunicamycin, or DMSO for 24 h after transfection of a luciferase plasmid containing various sizes of CHOP promoters.

anti-Apo2L/TRAIL receptor antibody (DR4, DR5, DcR1, or DcR2; eBioscience) were added. To assess nonspecific staining, phycoerythrin-labeled control IgG isotypes (eBioscience) were applied. After 30-min incubation on ice, cells were washed and 2×10^4 cells were analyzed by a FACSCalibur flow cytometer (Becton Dickinson).

Detection of Apoptosis

DNA fragmentation was quantified by the percentage of hypodiploid DNA (sub G_1). PC-3 and DU145 cells were treated with PBS containing 0.1% Triton X-100. Cells were then treated with RNase A (Sigma) and the nuclei were stained with propidium iodide (Sigma). Measurement and analyses were carried out as previously described (37). The DNA content was measured using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson). For all assays, 10,000 cells were counted.

siRNAs

The DR5 and LacZ siRNA sequences were previously described (34, 35). The XO siRNA sequences were as follows (sense and antisense, respectively): 5′-r(GCCCUUUGCUAUGGUGGAA)dTdT and 5′-r(UUCCACCAUAGCAAAGGGC)-dTdT (synthesized by Sigma). LacZ siRNA was used as a siRNA control. In brief, 1 d before transfection, PC-3 cells were seeded without antibiotics at a density of 30% to 40%. DR5, LacZ, and XO siRNA were transfected into cells using a modified Oligofectamine protocol (Invitrogen), in which the volume of Oligofectamine was reduced to one third of the recommended volume to limit toxic effects. Twenty-four hours after transfection, cells were treated with allopurinol and/or TRAIL for 24 h and then harvested.

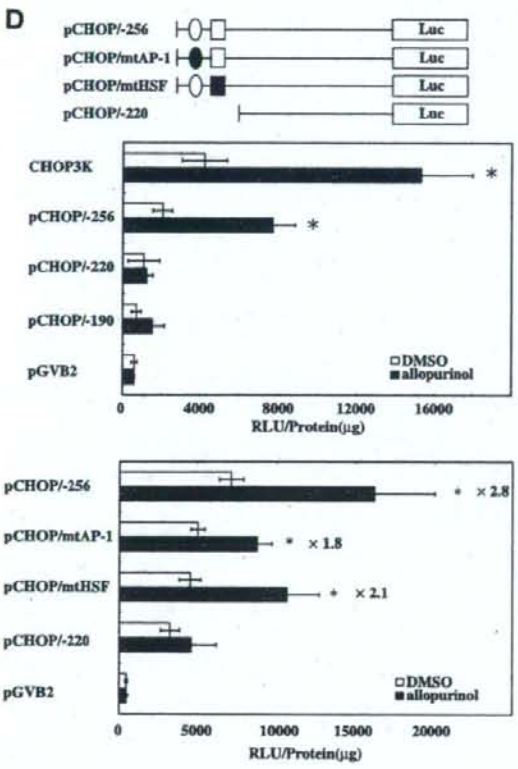

FIGURE 7 *Continued.* **D.** Structures of CHOP promoter-luciferase reporter plasmids. A circle and a square indicate AP-1 and HSF, respectively. Open circle and square, wild-type; closed circle and square, mutant. Cells were treated with 200 μmol/L allopurinol or DMSO for 24 h after transfection of a luciferase plasmid containing various CHOP promoters. pGVB2 is a vacant control plasmid. Columns, mean of triplicate experiments; bars, SD. *, $P < 0.05$.

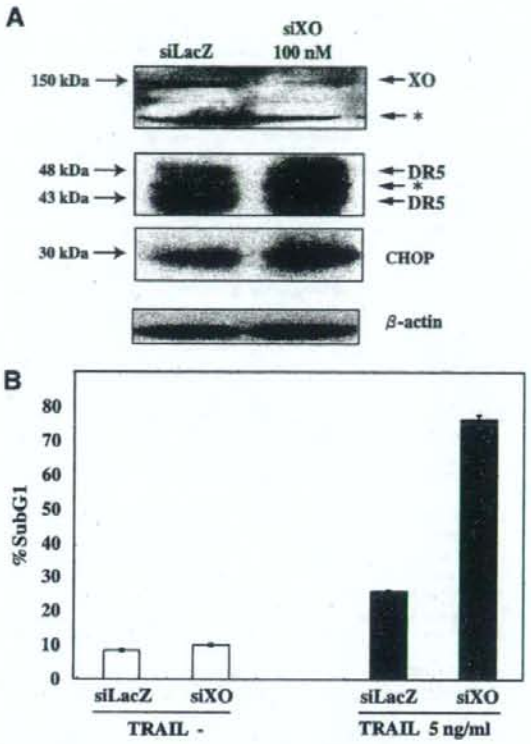

FIGURE 8. XO-specific siRNA up-regulates DR5 expression and enhances TRAIL-induced apoptosis in PC-3 cells. **A.** Western blot analysis showing the effects of XO siRNA or control siRNA transfections on XO expression. XO siRNA or control LacZ siRNA was transfected into PC-3 cells. Forty-eight hours after transfection, cells were analyzed by Western blotting. β-actin was used to ensure equal gel loading. Asterisks indicate nonspecific bands. The 48- and 43-kDa bands correspond to long and short DR5 isoforms, respectively. **B.** XO siRNA enhances TRAIL-induced apoptosis. PC-3 cells were treated with XO siRNA or LacZ siRNA. Twenty-four hours after transfection, cells were treated with or without TRAIL (5 ng/mL) for 24 h. Apoptosis was determined by FACS analysis of the DNA fragmentation of propidium iodide–stained nuclei as described in Materials and Methods. Data, mean of triplicate experiments; bars, SD.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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INTERNAL MEDICINE

Hypertension and Other Risk Factors for the Development of Kidney Cancer (Renal Cell Carcinoma) in a Japanese Population: Findings from the JACC Study

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ABSTRACT

Background: **Although the incidence of kidney cancer in Japan is lower than the rates in the other developed countries, there is no doubt that in Japan the incidence rate of kidney cancer has increased recently.**

Methods: **We evaluated the risk factors of kidney cancer using the database of the Japan Collaborative Cohort (JACC) study in 62,869 subjects (25,348 men and 37,521 women). Cox-proportional hazards model was used to determine age-and-sex adjusted relative risk.**

Results: **During the follow-up of 7.6 years, we identified 40 incident cases of kidney cancer (renal cell carcinoma). Hypertension and kidney disease were revealed as significant risk factors for kidney cancer. Both systolic and diastolic blood pressure showed a positive relation to the development of kidney cancer risk.**

Conclusion: **The present study suggests that hypertension and kidney disease may be associated with an increased risk for the development of kidney cancer.**

KEY WORDS

renal cell carcinoma, hypertension, kidney disease, risk factor, Japan

INTRODUCTION

The incidence of kidney cancer (renal cell carcinoma) is highest in Western Europe, Northern Europe and North America, intermediate in Japan, and low elsewhere in Asia[1,2]. Although the incidence of kidney cancer in Japan is lower than the rates in other developed countries, there is no doubt that the incidence has been increasing[3]. The incidence rates (persons per 100, 000) were 7.1 for men and 3.1 for women in 1997[4] while they were 8.2 for men and 3.6 for women in 2002[5].

Hypertension and obesity are risk factors for kidney cancer in Western countries [1,2,6]. Chow *et al*[7] reported that elevated blood pressure and higher body mass index increase the risk of kidney cancer.

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study) is a large prospective cohort

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Table 1. HR and 95% CI for kidney cancer according to medical history

Medical history of disease		Person-years	No. of cases	Age-and sex-adjusted HR (95% CI)
Hypertension	(−)	348,164	17	1.00 (reference)
	(+)	98,271	19	4.27 (2.07, 8.79)
Diabetes mellitus	(−)	414,718	30	1.00 (reference)
	(+)	22,019	4	1.72 (0.51, 5.79)
Kidney disease*	(−)	414,815	29	1.00 (reference)
	(+)	20,271	5	4.42 (1.68, 11.63)

HR: hazard ratio, 95% CI: 95% confidence interval, Kidney disease*: kidney diseases other than kidney cancer

study sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan[8]. Using data of this cohort study, we have already investigated risk factors for kidney cancer death in the Japanese population[9,10]. In these studies[9,10], medical history of hypertension was suggested to be a risk factor for kidney cancer death but obesity failed to show any meaningful relation to kidney cancer death. In Japan, patients with kidney cancer may be diagnosed in the early stage with renal imaging techniques such as ultrasonography and computer tomography[1]. Such incidental kidney cancer increased from 1980s and now accounts for 70-80% of all[1]. Most of the incidental kidney cancer cases may not have died from kidney cancer because most of the patients with incidental kidney cancer may have been saved by operations. Therefore, in the present study, we analyzed the incidence of kidney cancer to evaluate the risk factor for kidney cancer in the Japanese population.

SUBJECTS AND METHODS

The present study was part of the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study), a large prospective cohort study sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan[8]. Briefly, the original study population consisted of 46,465 men and 64,327 women aged 40 to 79 years in 45 areas of 19 prefectures in Japan. Enrolment began in 1988 and continued to 1990. Most subjects were recruited from the general population when undergoing routine health checks in the municipalities. The self-administered questionnaire addressed various health-related questions concerning medical histories, height and weight, health conditions and life-style habits such as smoking, drinking, diet, and physical exercise.

Body mass index (BMI) was calculated as the reported weight divided by the square of the reported height (kg/m^2). High BMI (BMI ≥ 25.0) was defined as obesity.

Informed consent for participants was obtained by signing the cover page of the questionnaire in 36 out of 45 areas while group consent was obtained from the community leader by explaining the aim of study and confidentiality of the data in 9 areas.

Follow-up surveys were conducted annually in order to determine the vital status of the participants. All deceased persons in the study areas and persons who moved out of the study areas were identified using the population registry with the permission of each municipality office. For logistical reasons, we discontinued the follow-up of subjects who moved out of their study area. However, incident cancer cases were ascertained only in 24 study areas (out of 45) in which cancer registries were available, where the mortality-incident rate deviated from 0.31 to 0.61 in men and from 0.15 to 0.53 in women[11].

The cancer registry system comprised reports from clinicians and death certificates; cancer cases with information from death certificate only (DCO) were included in incident cancer cases. The incidence including DCO from kidney cancer was identified by code C64 (i.e., renal cell carcinoma) in the ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th Revision). The end of the follow-up period for the cancer incidence survey was December 31, 1997 except one area (December 31, 1994). For analytical purposes, this study included the subjects who lived in study areas where cancer registries were available.

After excluding those with a medical history of kidney cancer, we had a total of 62,869 subjects (25,348 men and 37,521 women) for analysis.

This investigation was approved by the Ethical Boards of Nagoya University School of Medicine and Kyoto Prefectural University of Medicine.

All statistical analyses were conducted using the Statistical Analysis System (SAS) package. The hazard ratios (HRs) of kidney cancer incidence and 95% confidence intervals (95% CIs) were estimated with Cox's proportional hazard model. Age was treated as a continuous variable while indicator variables were used for other factors. The dose-dependent trend was tested by evaluating the regression coefficient when the three categories were treated as equally spaced numerical variables in Cox's model. P values of less than 0.05 were considered to be statistically significant.

RESULTS

During the follow-up of 7.6 (± 1.9) years, we identified 40 incident cases of kidney cancer (25 in men and 15 in women). Compared with women, men had a higher risk of kidney cancer (age-adjusted HR = 4.52, 95% CI: 2.28, 8.96), and the risk increased with age (sex-adjusted HR = 1.08 per 10-year increment, 95% CI: 1.05, 1.11) (not shown in the table).

Table 1 shows the age- and sex-adjusted relative risk of kidney cancer in relation to medical history. Hypertension (HR = 4.27, 95% CI: 2.07, 8.79) as well as kidney disease (HR = 4.42, 95% CI: 1.68, 11.63) was revealed as a significant risk factor for kidney cancer. Diabetes mellitus

Table 2. HR and 95% CI for kidney cancer according to blood pressure level

Medical history of disease		Person-years	No. of cases	Age-and sex-adjusted HR (95% CI)	p for trend
Systolic bloood pressure	139 mmHg or less	227,706	10	1.00 (reference)	
	140-149 mmHg	72,884	7	1.64 (0.57, 4.72)	
	150 mmHg or over	62,516	13	4.34 (1.75, 10.74)	<0.01
Diastolic bloood pressure	84 mmHg or less	254,463	11	1.00 (reference)	
	85-89 mmHg	34,860	4	2.00 (0.55, 7.27)	
	90 mmHg or over	70,629	15	4.82 (2.13, 10.93)	<0.01

HR: hazard ratio, 95% CI: 95% confidence interval

Table 3. HR and 95% CI for kidney cancer according to body mass index *

Medical history of disease		Person-years	No. of cases	Age-and sex-adjusted HR (95% CI)
Body mass index*	24.9 or less	388,434	30	1.00 (reference)
at the base line survey	25.0 or over	96,426	10	1.35 (0.66, 2.75)
Body mass index*	24.9 or less	416,416	32	1.00 (reference)
at the age of 20 years old	25.0 or over	64,531	8	1.66 (0.77, 3.61)

HR: hazard ratio, 95% CI: 95% confidence interval

Body mass index* : calculated as the weight divided by the square of height (Kg/m^2).

Table 4. HR and 95% CI for kidney cancer according to smoking , drinking and leisure time physical activity

Medical history of disease		Person-years	No. of cases	Age-and sex-adjusted HR (95% CI)	p for trend
Current smokers	(−)	333,065	25	1.00 (reference)	
	(+)	112,042	12	0.94 (0.42, 2.11)	
Current drinkers	(−)	251,516	16	1.00 (reference)	
	(+)	201,516	20	1.10 (0.49, 2.46)	
Leisure time	Seldom	318,537	30	1.00 (reference)	
physical activity	1-2 hours/week	68,786	4	0.43 (0.13-1.43)	
	3-4 hours/week or more	49,796	4	0.66 (0.23-1.92)	0.24

HR: hazard ratio, 95% CI: 95% confidence interval

showed an HR greater than the unity (HR = 1.72, 95% CI: 0.51, 5.79), but it was not a significant risk factor.

Table 2 illustrates the age- and sex-adjusted HRs for the development of kidney cancer in relation to blood pressure level. Systolic blood pressure was positively associated with the risk of kidney cancer. Compared with 139 mmHg or less, HRs were 1.64 (95% CI: 0.57, 4.72) for 140-149 mmHg and 4.34 (95% CI: 1.75, 10.74) for 150 mmHg or over. Like systolic blood pressure, diastolic blood pressure also showed a positive relation to the kidney cancer risk (p for trend < 0.01). Compared with 84 mmHg or less, HRs were 2.00 (95% CI: 0.55, 7.27) for 85-89 mmHg and 4.82 (95% CI: 2.13, 10.93) for 90 mmHg or over.

Table 3 presents the age- and sex-adjusted HRs for the development of kidney cancer in relation to body mass index (BMI). Compared with low BMI (24.9 or less), obesity with high BMI (25.0 or over) showed increased HRs for the development of kidney cancer both at the baseline survey (HR = 1.35, 95% CI: 0.66, 2.75) and at the age of 20 years old (HR = 1.66, 95% CI = 0.77, 3.61), but they were not statistically significant.

Table 4 shows the age- and sex-adjusted HRs for the development of kidney cancer in relation to life style factors such as smoking, drinking and leisure time physical activity. There were no meaningful associations between life-style factors and the risk of kidney cancer.

DISCUSSION

Hypertension [1,2,6,7,12,13,14] as well as anti-hypertensive medication[1,2,6,12,13,14] has been reported to be a risk factor for kidney cancer in Western countries. Although it is difficult to distinguish the effects of hypertension from those of anti-hypertensive medications on the risk of kidney cancer in epidemiological studies[15], hypertension is an established risk factor for kidney cancer in Western populations[1,2,6]. In contrast with Western populations, there are few reports on the association between hypertension and kidney cancer in Asian populations[9,10,16,17]. Grove *et al*[16] reported that blood pressure was positively associated with the incidence of kid-

ney caner among Japanese American in Hawaii while Choi *et al*[17] reported that hypertension was an independent risk factor for kidney cancer death in Korean men. In our previous studies[9,10], hypertension was suggested to increase the risk of kidney cancer death in the Japanese population although the risk failed to reach a statistically significant level in one study[10]. In the present study, medical history of hypertension increased the risk of developing kidney cancer in Japanese peoples in Japan. Furthermore, both systolic and diastolic blood pressure levels were positively associated with the incidence of kidney cancer. These findings are consistent with the result of the study by Grove *et al*[16], where there was a positive association between blood pressures and risk of kidney cancer among Japanese immigrants and their descendants in Hawaii. Since we had no information about the antihypertensive treatment in the present study, we can not deny the possibility that subjects with higher blood pressures took more anti-hypertensive drugs. Among antihypertensive drugs, diuretics are the most likely perpetrators to increase the risk of kidney cancer[18]. Additional studies will be needed to distinguish the effects of hypertension from those of anti-hypertensive medications on the risk of developing kidney cancer in the Japanese population.

The mechanisms of how hypertension increases the risk of kidney cancer may be explained in the following way. Insulin resistance is the metabolic manifestation of systemic inflammatory state with oxidative stress and contributes a risk factor for numerous malignancies[19]. Insulin resistance and hyperinsulinemia are common in obesity and may play a role in obesity-related hypertension[20]. However, insulin resistance is also seen in non-obese hypertensives[20]. Thus, insulin resistance, which increases the risk of cancer, is seen among hypertensive subjects regardless of obesity. Insulin resistance leads to elevated levels of insulin-like growth factor type 1, which is associated with increased risk of cancer[19].

Kidney diseases such as kidney infections, kidney stones, and kidney cysts are risk factors for kidney cancer in western countries[1,2]. Parkers *et al*[21] reported a positive association of urinary tract infection with the development of kidney cancer. In the present study, a medical history of kidney diseases is associated with an increased risk of developing kidney cancer although a medical history of kidney diseases failed to show an increased risk of kidney cancer death in our previous study[9]. Inconsistent findings in our two studies may be explained by the following possibility. The diagnosis of kidney cancer is often delayed after the disease is advanced because small localized tumors rarely produce symptoms such as hematuria, abdominal pain, and a palpable mass in the flank or abdomen[6]. In Japan, incidental kidney cancer may be found in the early stage among the patients with kidney diseases because they are examined by renal imaging techniques for their kidney diseases while those without kidney diseases had little chance to have an examination of their kidney with renal imaging techniques. Therefore, patients with kidney disease may be saved by an operation in the early stage of kidney cancer while subjects without kidney disease may die from kidney cancer because their cancer is detected in the advance stage when most patients have metastasis to other organs.

Obesity[1,2,6,7,12,15,22] is reported as a risk factor for kidney cancer in western countries. Insulin resistance, which is common in obesity[20], contributes a risk factor for numerous cancers[19]. However, like our previous studies[9,10], obesity (BMI ≥ 25.0) failed to show a significantly increased risk of kidney cancer, which may be explained by the fact that the number of kidney cancer cases in the present study was very small. In addition, the low prevalence of obesity in the participants could be another explanation why obesity was not a risk factor for kidney cancer death in this study

Diabetes mellitus increases the risk of kidney cancer in some studies[21,23,24], but it is controversial whether diabetes mellitus increases the risk of kidney caner[2]. In our previous studies[9,10], diabetes mellitus showed a non-statistically significant excess risk of kidney cancer death in the former study[9] while significantly increased risk due to diabetes mellitus was found in the latter study[10]. In the present study, diabetes mellitus showed an HR greater than the unity, but it was not a significant risk factor for kidney cancer.

In Western countries, smoking[1,2,6,7,18] has been reported to increase the risk of kidney cancer while alcohol drinking[1,2,25] and high physical activity[1,2,26] have been suggested to reduce the risk of kidney cancer. However, like our previous studies[9,10], we failed to show any meaningful association between lifestyle factors and kidney cancer risk in the present study.

Our study has some limitations. First, the number of kidney cancer cases was very small in spite of a large scale study. Second, we did not evaluate kidney cancer risk in men and women separately after controlling other factors because the number of kidney cancer cases was small. Third, we had no information about the antihypertensive treatment. Fourth, blood pressure in the present study was self-reported blood pressure. However, most of subjects were asked to answer the self-administered questionnaire at routine health checks in the municipalities. Last, self-reported weight and height in self-administered questionnaires were used to determine BMI.

In summary, the present study showed that medical history of hypertension, high systolic blood pressure level, high diastolic blood pressure level, and medical history of kidney disease may increase the risk of kidney cancer. To our best knowledge, this is the first report to evaluate the risk factor for developing kidney cancer in a large prospective cohort study in Japan. However, further studies will be needed to evaluate the risk of kidney cancer in relation to hypertension in Japanese peoples because the number of incident kidney cancer cases was small in the present study.

Although cohort studies of risk are the best available substitutes for a true experiment, cohort studies need a large number of people under observation for a long time [27]. In addition, the incidence of kidney cancer is very low in Japan (i.e., 7.1 persons per 100, 000 for men and 3.1 persons for women in 1997[4]; 8.2 persons per 100, 000 for men and 3.6 persons for women in 2002[5]) although it has been increasing[3]. Therefore, we identified only 40 incident cases of kidney cancer in spite of a large scale cohort study. Since investigators can identify cases unconstrained by the natural frequency of disease in case-control studies[27], additional case-control studies provided other information such as antihypertensive drugs may be useful to confirm the result of the present study.

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