厚生労働科学研究費補助金

循環器疾患等総合研究事業

糖尿病性腎症の寛解を目指したチーム医療による集約的治療に関する研究

（臨床研究実施チームの整備）

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（臨床研究実施チームの整備）

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研究要旨　日本人 2 型糖尿病性顕性腎症に対する、蛋白制限食の有効性について、多施設共同研究によるランダマイズコントロール（RCT）された、5 年間の検証研究がなされた。前観察期（蛋白摂取 1.2g/kg/day 以上）の 3 ヶ月後、蛋白制限食（A）群（蛋白指示量；0.8g/kg/day）と通常蛋白食（B）群（蛋白指示量；1.2g/kg/day）の 2 群に RCT され、観察期として 5 年間 follow された。本研究では、観察期 24 ヶ月までの症例を対象とした。調査ポイントは、前観察期として-2、-1、0 ヶ月及び観察期として 3、6、12、24 ヶ月目とした。平均を算出し解析した。A 群：47 例、B 群：46 例であった。栄養調査上、前観察期では A 群と B 群の間で蛋白摂取量に有意差を認めず、観察期では、A 群（蛋白摂取量；0.92g/kg）は B 群（蛋白摂取量；1.11g/kg）に比較し、有意に低値であり、食事調査上からは指導が的確に行われたと考えられる。しかし、観察期で蛋白以外の栄養素摂取（カルシウム、ビタミン B_1）の、また酸化ストレス関連栄養素（ビタミン E, B_6、カロテン、葉酸）の摂取量が、A 群で B 群のそれらに比較し、有意に低値であり、かつ A 群では食事摂取基準（正常人）に満たない栄養素（カルシウム、ビタミン E, B_1, B_6、カロテン）が多かった。これらは糖尿病性顕性腎症の栄養指導法に大きな問題を残し、検討すべき課題となった。また今年度より開始される基幹研究（糖尿病性腎症の寛解を目指したチーム医療による集約的治療）のプロトコール作成に活かすことが出来た。

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A. 採択された研究事業での研究概要

日本人 2 型糖尿病性顕性腎症に対する、蛋白制限食の有効性について、多施設共同研究によるランダマイズコントロール（RCT）された、5 年間の検証研究が本研究の基幹研究班によりなされている。前観察期（蛋白摂取 1.2g/kg/day 以上）の 3 ヶ月後、蛋白制限食（A）群（蛋白指示量；0.8g/kg/day）と通常蛋白食（B）群（蛋白指示量；1.2g/kg/day）の 2 群に RCT され、観察期として 5 年間 follow されている。本研究では、観察期 24 ヶ月までの症例を対象とした。調査ポイントは、前観察期として-2、-1、0 ヶ月及び観察期として 3、6、12、24 ヶ月目とし、平均を算出し解析した。A 群：47 例、B 群：46 例であった。栄養調査上、前観察期では A 群と B 群の間

で蛋白摂取量に有意差を認めず、観察期では、A群（蛋白摂取量；0.92g/kg）はB群（蛋白摂取量；1.11g/kg）に比較し、有意に低値であり、食事調査上からは指導が的確に行われたと考えられた。しかし、観察期で蛋白以外の栄養素摂取（カルシウム、ビタミンB_1）の、また酸化ストレス関連栄養素（ビタミンE,B_6、カロテン、葉酸）の摂取量が、A群でB群のそれらに比較し、有意に低値であり、栄養所要量に満たないものが多かった。これらは、2年間の平均であるが、糖尿病性顕性腎症の栄養指導法に大きな問題を残し、検討すべき課題である。

今回の基幹研究は、医師と糖尿病療養指導士が参加して、糖尿病性腎症に対する集約的治療を目指すものである。そこで当臨床研究チームは①食事栄養指導における栄養素摂取の実態を把握し、病態との関連性を検討する。②集約的治療による酸化ストレス関連マーカーの変化と病態との関連性を検討する。以上より食事・栄養の面から指導法も含めた指針を提供する。

B. 採択された研究事業での研究実績

今回は前観察期3ヶ月及び観察期（蛋白制限食（A）群又は通常蛋白食（B）群）24ヶ月の期間の検討を行った。

食事摂取調査からは、前観察期では、A群とB群の食事蛋白摂取量に有意差を認めなかった。観察期のA群の摂取蛋白量は0.92g/kg、B群の摂取蛋白量は1.11g/kgで、有意差を認め、食事調査上からは、指導が的確に行われたと推察される。

①前観察期と観察期の間の、各群の摂取量の変化について検討した。A群において、糖質摂取量は前観察期に比して観察期で有意に増加し（$p=0.006$）、カルシウム摂取量は減少した（$p<0.001$）。

②蛋白以外の栄養摂取量について、前観察期、観察期におけるA群とB群間の比較を行った。前観察期ではビタミンB_1摂取量がB群に比較しA群で有意に低かった（$p=0.008$）。一方、観察期では、蛋白質（$p<0.001$）以外、ビタミンE（$p=0.045$）、B_1（$p<0.001$）、B_6（$p=0.007$）、カロテン（$p=0.013$）、カルシウム（$p<0.001$）、葉酸（$p<0.001$）の各摂取量が、B群に比較してA群で有意に低かった。

③各栄養素の摂取量を、栄養所要量（現：食事摂取基準（正常人））から検討すると、図1に示すように、蛋白制限食群（A群）のカルシウム、ビタミンE, B_1, B_6及びカロテンの摂取量は、栄養所要量(RDA)（現：食事摂取基準）に満たなかった。

（倫理面への配慮）

本研究は、基幹研究の主任研究者（岡山大大学院医学歯学総合研究科：槇野博史教授）及び分担研究者（当研究申請者）施設での倫理委員会承認の上、文書による説明と同意を得て行う。

C. 考察

今回検討項目としたビタミンB_1摂取量は、前観察期でもA群で低値であった。これは、前観察期3ヶ月間、通常蛋白食を指導しても、それ以前に糖尿病性腎症のいわゆる「低蛋白食」を指導されており、その影響があった可能性もある。今回の成績で驚くことに、A群観察期（指示蛋白制限0.8g/kg施行期）で、栄養調査上、ビタミンE,B_1,B_6、カロテン、葉酸、更にカルシウムの摂取量が、B群（蛋白摂取指示量；1.2g/kg）のそれらに比較し、有意に低値であり、かつ食事摂取基準に達していなかったことである。これは、今後の栄養指導法に問題を残したと考えられる。2年間の平均としての成績であるが、これらの栄養素の長期の低摂取が、通常食を摂取した場合に比べ、生体にどのような影響があるのか、今後の検討が必要であろう。この成果を今

年度より開始される「糖尿病性腎症の寛解を目指したチーム医療による集約的治療」のプロトコール作成に是非活かすべきと考える。

食事調査よりの栄養素摂取研究は重要であるにも関わらず、方法論や解析方法（交絡因子の関与）にも問題があり、今後の新展開が必要である。

D. 健康危険情報

特になし

E. その他実施した臨床研究・治験の概要及び実績

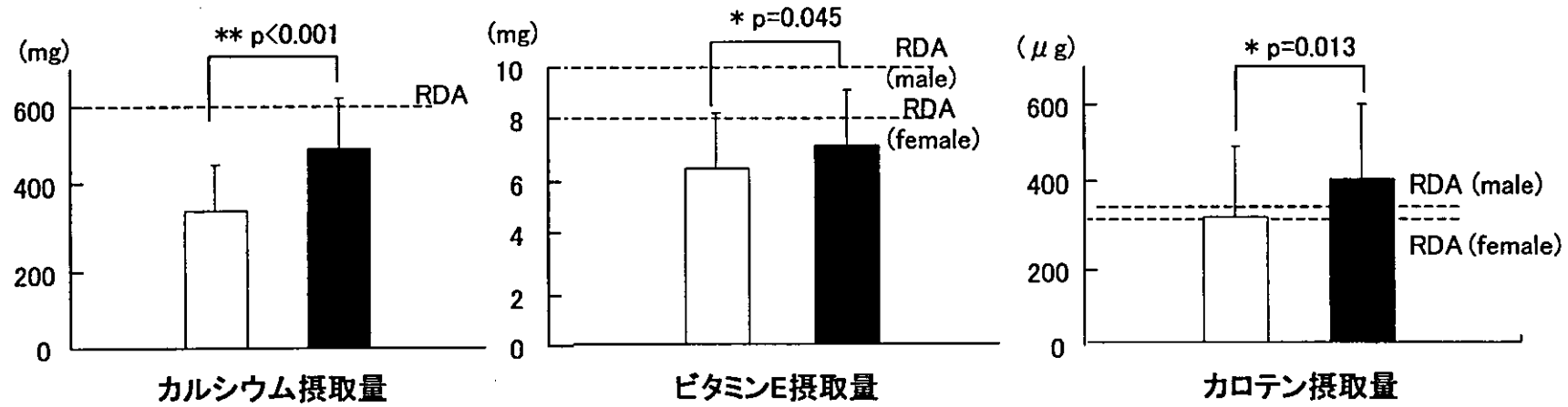
①CS-866DM による顕性蛋白尿を呈する２型糖尿病に伴う糖尿病性腎症患者を対象とした二重盲検比較試験　４症例（現時点：２症例）

②ピタバスタチンの腎機能障害患者における薬物動態試験（単回および７日間連続投与試験）　腎機能正常群６症例、腎機能障害群６症例（現時点：腎機能正常群１症例、腎機能障害群１症例）

《図1》

観察期のA群とB群の比較

□ A群（蛋白制限食群 0.8g/kg/day), n=47　　■ B群（通常蛋白食群 1.2g/kg/day), n=46



※Mann-Whitney検定

(M±SD)

研究成果の刊行に関する一覧表

雑誌

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Original Article

Renoprotective Effect of Losartan in Comparison to Amlodipine in Patients with Chronic Kidney Disease and Hypertension—a Report of the Japanese Losartan Therapy Intended for the Global Renal Protection in Hypertensive Patients (JLIGHT) Study

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A 12-month, multicenter (57 clinical institutions), randomized, open-labeled trial was undertaken to compare the efficacy of the angiotensin II receptor antagonist losartan and the calcium channel blocker amlodipine in patients with proteinuric chronic kidney disease (CKD) and hypertension. A total of 117 patients (79, chronic glomerulonephritis; 14, diabetic nephropathy; 24, other CKD) were randomly allocated into two treatment groups. Losartan and amlodipine exerted the same efficacy for blood pressure (BP) control; however, losartan significantly reduced the 24-h urinary protein excretion at months 3, 6, and 12, with the reduction of 20.7%, 35.2%, 35.8%, whereas amlodipine did not change the amount of proteinuria over the 12-month study period. When patients were stratified into groups according to the level of BP control at 3 months, the reduction in urinary protein excretion by losartan was evident in the group for which a BP of <140/90 mmHg was achieved, as well as in the group for which the goal BP (<130/85 mmHg) for treatment of CKD was not achieved. When patients were stratified according to baseline urinary protein excretion, those with ≥2 g/day showed a reduction in proteinuria by losartan of 23.3%, 39.4%, and 47.9% at months 3, 6, and 12, and those with <2 g/day showed a reduction of 18.5% and 31.2% at months 3 and 6, respectively. No fatal adverse

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* Coordinating Committee. # Independent Data-Monitoring Committee.

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events were experienced in either drug group. We conclude that losartan reduced proteinuria in patients with CKD and hypertension. This positive effect may contribute to the renal protective benefit of losartan, and is beyond the magnitude of BP control. (*Hypertens Res* 2004; 27: 21–30)

Key Words: losartan, angiotensin, proteinuria, hypertension, renoprotection

Introduction

On the basis of understanding the role of angiotensin II in circulation and renal functions, the relevance of intervention of the renin-angiotensin system (RAS) for therapy of hypertension and kidney diseases has so far been extensively discussed (*1*, *2*). High blood pressure (BP) strongly affects the structure and functions of nephrons, and inversely, impaired renal function elevates the systemic BP level in patients with kidney diseases. Angiotensin converting enzyme (ACE) inhibitors are now one of the most frequently used drugs for hypertension, and a number of evidences are available with regard to the effect of ACE inhibition to ameliorate kidney diseases, especially proteinuria as a symptom (*3*). Indeed, in many clinical studies dealing with kidney diseases, proteinuria has been adopted as a surrogate endpoint, because proteinuria is not merely a marker of permselectivity of the glomerular membrane, but is toxic to the kidney *per se*, and plays a key role in the progression of kidney diseases, eventually leading to end-stage renal disease (ESRD) (*4–7*).

With reference to the effect of ACE inhibitors, the use of angiotensin II receptor antagonists for the treatment of kidney diseases has also been discussed. The RENAAL study, an international multicenter clinical trial of the angiotensin II receptor antagonist losartan, was published in 2001 (*8*). This trial studied the effect of losartan in patients with type 2 diabetic nephropathy. The results clearly demonstrated that losartan retarded the elevation of serum creatinine and decreased the rate of onset of ESRD. On the other hand, the effects of intervention of the actions of angiotensin II in patients with non-diabetic chronic kidney disease (CKD) and hypertension has been still a subject of debate with regard to relation to BP lowering effect. Any pharmacotherapy to lower BP may be effective for protection of renal functions; however, whether blockade of angiotensin II receptors confers renal protection in excess of that due to BP control has not been clearly answered. There is thus need of accumulation of evidences of comparative study with other classes of antihypertensive drugs in patients with CKD and hypertension. For this reason, we have performed a 12-month study comparing the effects of the angiotensin II receptor antagonist losartan and the calcium channel blocker amlodipine. A portion of the results were previously disclosed as an interim report at 3 months (*9*) with the full analysis set (FAS) (*10*). We here report our final results based on the final selection of patients by the Coordinating Committee. Our findings show that, although losartan and amlodipine exerted the same degree of BP control, only losartan induced a significant reduction in urinary protein excretion over the 12-month observation period.

Methods

This study was a 12-month, multicenter, randomized, open-labeled, clinical trial designed to compare the effect of the angiotensin II receptor antagonist losartan and the calcium channel blocker amlodipine to reduce proteinuria in patients with CKD and hypertension. Fifty-seven affiliated clinics in Japan contributed to this study. The overall design of the study has been described previously in an interim report presented at 3 months (*9*). Males and female outpatients, aged 20–74 years, who had CKD and hypertension and who met the following criteria during the 8-week pretreatment screening period were eligible for the study:

1) CKD: serum creatinine (Scr) levels of $1.5 \leq Scr < 3.0$ mg/dl in males of body weight (BW) ≥ 60 kg, and of $1.3 \leq Scr < 3.0$ mg/dl in females, or males of BW < 60 kg.

2) Hypertension: systolic BP (SBP) ≥ 140 mmHg or diastolic BP (DBP) ≥ 90 mmHg as measured in a sitting position at least two separate times at their visits to clinics.

3) Proteinuria: urinary protein excretion of ≥ 0.5 g/day.

The overview of study design is shown in Fig. 1. The randomization method was modified by dynamic balancing for Scr, the 24-h urinary protein excretion that was measured at the time of registration, and presence or absence of diabetic nephropathy, so that patients were allocated to the two groups avoiding significant difference of baseline characteristics in average. Patients of the two groups received either losartan 25 mg as a starting dose to up to 100 mg once daily, or amlodipine 2.5 mg as a starting dose to up to 5 mg once daily, respectively. However, in cases in which a patient's compliance was judged by investigator(s) to be sufficiently good for the administration of a higher dose, either 50 mg of losartan or 5 mg of amlodipine was adopted as a starting dose.

The target BP was $< 130/85$ mmHg, and patients were not allowed combination therapy with other antihypertensive agents during the first 3 months. However, after 3 months, if a BP of $< 130/85$ mmHg was not achieved, antihypertensive combination therapy with α-blockers, β-blockers, α/β-blockers, diuretics (excepting potassium-sparing diuretics), and other calcium channel blockers were considered as appropriate. Guidance was given to patients to maintain their usual diet, especially for those under dietary restrictions. The study protocol was reviewed and approved by the Institutional Review Boards of all clinics contributing to the study. Written informed consent was obtained from all enrolled pa-

Table 1. Baseline Characteristics of Patients Enrolled in the Study

	Losartan group	Amlodipine group	*p* value
N	58	59	
Age (years)	55.7±13.6	57.5±11.9	NS*
Male/female	36/22	41/18	NS†
BMI (kg/m²)	23.9±3.7	22.9±3.2	NS*
Systolic BP (mmHg)	156.5±12.2	155.4±13.5	NS*
Diastolic BP (mmHg)	94.0±9.2	93.5±8.6	NS*
Serum creatinine (mg/dl)	2.04±0.48	1.97±0.52	NS*
Urinary protein (g/day)	2.85±2.65	2.50±2.07	NS*
Serum albumin (g/dl)	3.79±0.48	3.80±0.47	NS*
Diagnoses (No. of patients)			
Chronic glomerulonephritis	38 (11#)	41 (12#)	
Diabetic nephropathy	7	7	
Hypertensive nephrosclerosis	11	9	
Tubulointerstitial nephritis	1	0	
Polycystic kidney disease	1	0	
Renal amyloidosis	0	1	
Castleman's disease	0	1	

Mean±SD. * Unpaired *t*-test; † Fisher's exact test. # IgA nephropathy. BMI, body mass index; BP, blood pressure.

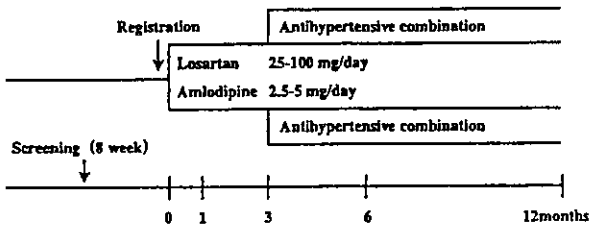

Fig. 1. *Study design for treatment of patients with proteinuric CKD and hypertension. Antihypertensive combination therapy was allowed after the first 3 months, if necessary. For this alternation, the target goal BP setting was <130/85 mmHg.*

tients.

Exclusion criteria were as follows:

1) DBP≥120 mmHg.
2) Renovascular hypertension or endocrine hypertension.
3) BP control treatment with antihypertensive agent(s).
4) Patients in whom antianxiety drugs could not be discontinued.
5) Pregnancy, possibility of pregnancy, or in a period of lactation.
6) Patients that the chief investigator judged not to be eligible.

BP was measured at patients' visit to the clinic with the patient in a sitting position.

A 24-h urine collection was performed from 8:00 AM of the day before to 8:00 AM of the day of the clinic visit, and was used to obtain the 24-h urine volume, urinary protein excretion, urinary creatinine level, and the amount of sodium excretion. The creatinine clearance (Ccr) was calculated as $Ccr = Ucr \times V/Scr \times 1.73/A$, where Ccr is the creatinine clearance (ml/min), Ucr is the urinary creatine (mg/dl), V is the urine volume (ml/min), Scr is the serum creatine (mg/dl), and A is the body surface area. The rate of renal impairment as a function of time was expressed with a reciprocal slope of Scr (1/Scr).

Protein intake was estimated by measurement of urea nitrogen plus protein concentration using the following formula: Protein intake (g/day)=[urinary urea nitrogen (g/day)+0.031(g)×BW(kg)]×6.25+urinary protein excretion (g/day) (*11*). Sodium chloride (NaCl) intake was measured by NaCl concentrations in the collected urine using the following formula: NaCl intake (g/day)=urinary sodium excretion (mEq/day)/17.

All values were expressed as the mean±SD. The baseline characteristics of the enrolled patients were tested for comparability between the losartan group and the amlodipine group using unpaired *t*-test or Fisher's exact test. The differences in changes in SBP and DBP between the two groups were tested by repeated-measures analysis of variance with treatment effect, period effect, and the interaction between treatment and period effect. Changes in urinary protein excretion, Scr, and Ccr within each group were analyzed by paired *t*-test. Unpaired *t*-test was used to compare the percent changes of urinary protein excretion, Scr, and Ccr between the losartan group and the amlodipine group. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

In all patients enrolled during the term from December 1999

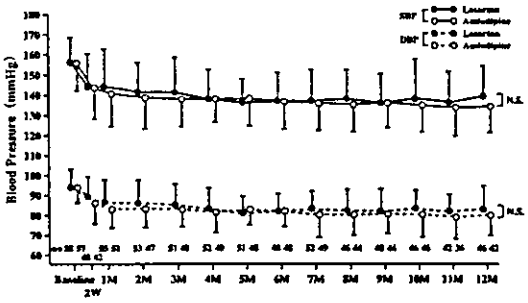

Fig. 2. *SBP and DBP changes (mmHg) throughout 12 months in groups treated with losartan and amlodipine. Circles and bars indicate the mean and SD. SBP and DBP were not significantly different between the losartan and amlodipine groups.*

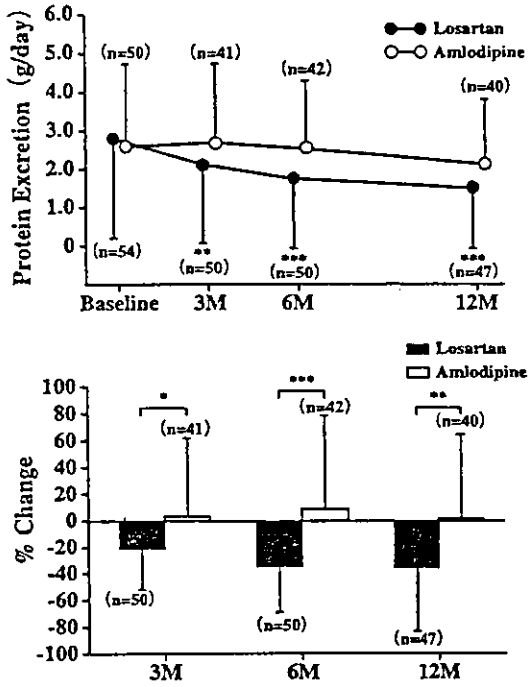

Fig. 3. *Changes in 24-h urinary protein excretion (upper panel) and respective percent changes (lower panel) from baseline. Circles and bars indicate the mean and SD.* * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

to March 2002, 117 patients (58 for losartan and 59 for amlodipine) were eligible, as their baseline characteristics are shown in Table 1. A large number of patients were diagnosed with chronic glomerulonephritis, including IgA nephropathy. Patients with diabetic nephropathy and hypertensive nephrosclerosis were also included. The characteristics of the two treatment groups were similar. Forty-seven patients in the losartan group and 40 patients in the amlodipine group completed the 12-month study for measurement of urinary protein endpoint. The dietary compliance assessment of 24-h urinary urea nitrogen plus proteins and sodium showed that, there was no significant difference in total protein and NaCl intake between the two drug treatment groups at baseline and no change from baseline to month 3, as reported previously (*9*). At month 12, again, there was no change from baseline and therefore no difference between the losartan group and the amlodipine group in protein intake or NaCl intake (protein [g/day]: losartan, 50.7±19.7; amlodipine, 53.5±17.0; NaCl [g/day]: losartan, 8.0±3.8; amlodipine, 9.6±3.5).

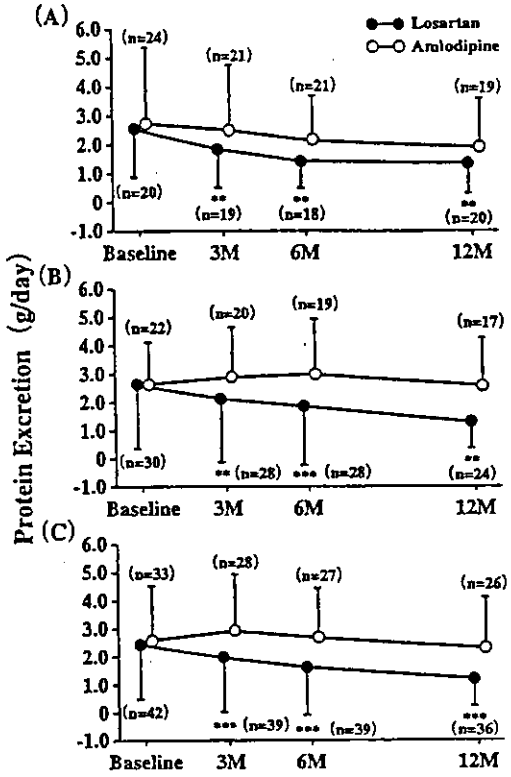

Fig. 4. *Changes in urinary protein excretion in patients stratified in response to BP control measured at month 3. (A) BP<140/90 mmHg. (B) BP≥140/90 mmHg. (C) BP≥130/85 mmHg. Note that patients in group C are included in either the group A or B because of respective BP ranges, as a consequence. Circles and bars indicate the mean and SD.* ** $p<0.01$, *** $p<0.001$.

The BP-lowering effect, in both systole (SBP) and diastole (DBP), was similar with losartan and amlodipine. Figure 2 shows changes in SBP and DBP measured at week 2 and at every month. In the losartan group, SBP was reduced from 156.5±12.2 mmHg at baseline to 139.5±14.8 mmHg at month 12 (−11.3±9.2%), and DBP from 94.0±9.2 mmHg at baseline to 83.0±11.7 mmHg at month 12 (−12.2±10.8%), and in the amlodipine group, the reduction in SBP

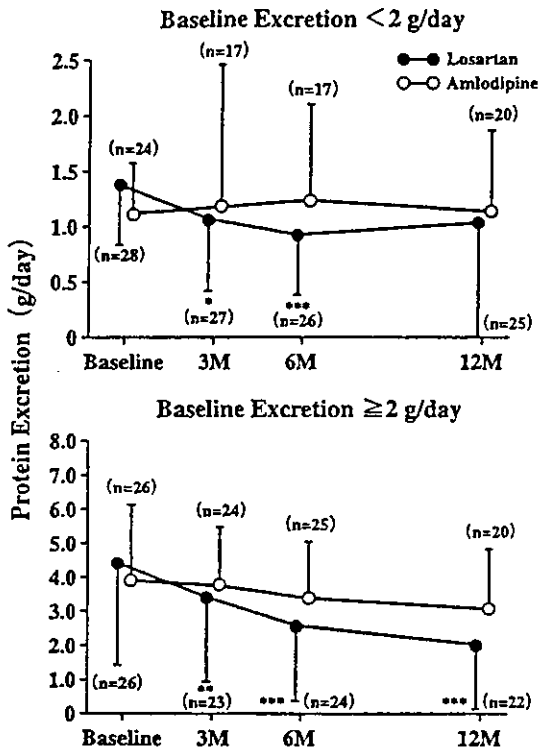

Fig. 5. *Changes in urinary protein excretion from baseline in patients stratified into two groups showing proteinuria of <2 g/day (upper panel) and ≥2 g/day (lower panel) as measured at baseline. Circles and bars indicate the mean and SD.* * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

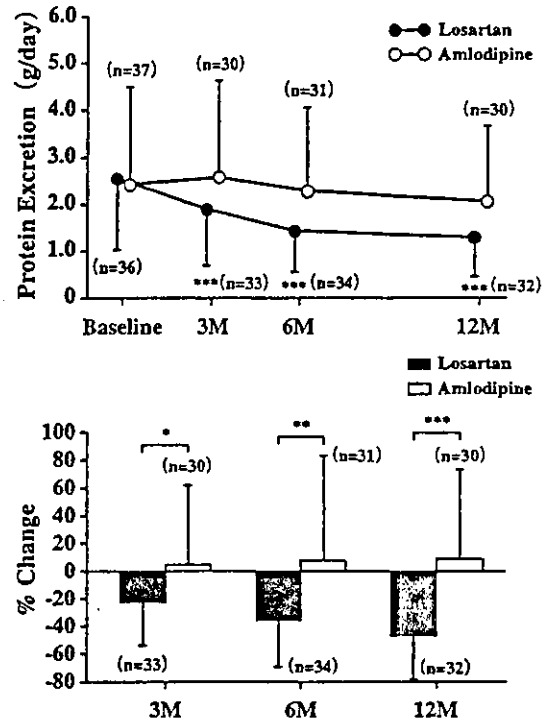

Fig. 6. *Changes in urinary protein excretion (upper panel) and respective percent changes (lower panel) in patients with chronic glomerulonephritis. Circles and bars indicate the mean and SD.* * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

was from 155.7±13.6 mmHg at baseline to 134.3±13.1 mmHg at month 12 (−12.7±10.0%), and that of DBP was from 94.1±7.9 mmHg at baseline to 79.7±10.1 mmHg at month 12 (−15.1±12.5%), respectively.

However, urinary protein excretion was significantly reduced only in the losartan group. The upper panel of Fig. 3 shows the change in urinary protein excretion and the lower panel shows the percent change from the respective baselines. The apparent changes in percent were −20.7%, −35.2%, and −35.8% at months 3, 6, and 12, respectively. We then analyzed the relationship between BP control and reduction of proteinuria in patients treated with losartan.

The responsiveness to the drug was assessed by BP measured at month 3. In this analysis, patients whose BP was controlled to <140/90 mmHg as well as those whose BP was not controlled at month 3 showed a statistically significant reduction in urinary protein excretion from baseline at each of months 3, 6, and 12. Although the JNC-VI guidelines recommend a BP goal of <130/85 mmHg for hypertensive patients with CKD (*12*), patients in whom this goal was not achieved still showed a statistically significant reduction in urinary protein excretion by losartan (Fig. 4). In the losartan group with a BP of <130/85 mmHg, there was an apparent reduction in urinary protein excretion, but without statistical significance.

Although at baseline there was no statistically significant difference between treatment groups in the ratio of males to females (Table 1), the number of female patients in the amlodipine group decreased during the study. However, in the losartan group, changes in proteinuria were almost comparable between males and females: −21.0% ($n=31$) and −20.2% ($n=19$) at month 3, −35.5% ($n=31$) and −34.6% ($n=19$) at month 6, and −35.2% ($n=29$) and −36.9% ($n=18$) at month 12 in males and females, respectively. Likewise, although no effect was observed with amlodipine, changes in the amount of proteinuria in males and females were +7.1% (n=31) and −8.0% ($n=10$) at month 3, +13.6% ($n=30$) and −4.6% ($n=12$) at month 6, and −1.5% ($n=30$) and +10.6% ($n=10$) at month 12, respectively.

In order to examine whether the magnitude of proteinuria affected the result of treatments with losartan and amlodipine, we stratified patients into two subgroups: those with proteinuria <1 g/day and those with proteinuria ≥1 g/day at baseline. In these subgroups, the change in urinary protein excretion from baseline was not significantly different between the losartan group and the amlodipine group. We next stratified patients with proteinuria levels of <2 g/day and

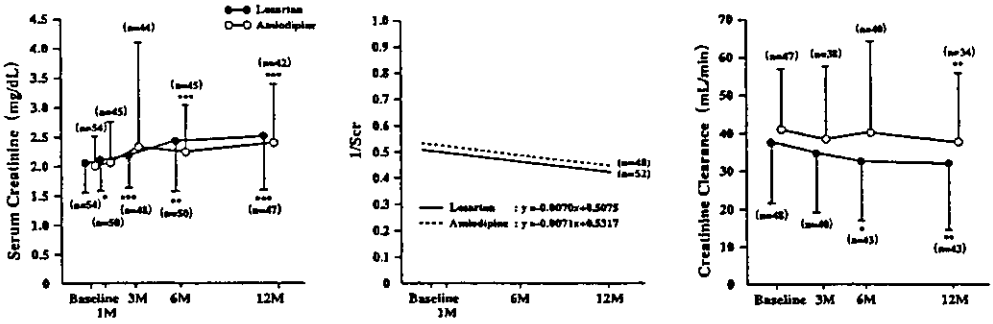

Fig. 7. *Changes in Scr (left panel), 1/Scr (middle panel), and creatinine clearance (right panel) in patients treated with losartan for Scr and 1/Scr. Circles and bars indicate the mean and SD.* * $p<0.05$, ** $p<0.01$, *** $p<0.001$. *There was no difference for the slope of 1/Scr between the losartan and amlodipine group.*

≥2 g/day at baseline. As shown in Fig. 5, the reduction in urinary protein excretion was evident in losartan groups of both <2 g/day and ≥2 g/day. Again, amlodipine did not significantly reduce urinary protein excretion in both groups of <2 g/day and ≥2 g/day.

With respect to the diagnosis of patients, 38 patients in the losartan group and 41 in the amlodipine group had chronic glomerulonephritis, and 7 in the losartan group and 7 in the amlodipine group had diabetic nephropathy. Analysis of the patients with diabetic nephropathy revealed an apparent decrease from baseline in urinary protein excretion in the two treatment groups, with no statistically significant difference between the groups (data not shown). Analysis of the subgroup with chronic glomerulonephritis exhibited a statistically significant reduction in proteinuria in the losartan group at months 3, 6, and 12. Because amlodipine did not reduce proteinuria in patients with chronic glomerulonephritis, there was a prominent difference in the percent reduction in urinary protein excretion from baseline between the two treatment groups (Fig. 6).

Changes in Ccr and Scr and the slope of 1/Scr did not differ between the two treatment groups. Scr slightly increased from the baseline to month 3 in both groups. Ccr showed a tendency of decline (Fig. 7).

Adverse events considered to be possibly related to the study were reported for increases in aspartate aminotransferase (AST; GOT) (2 cases), alanine aminotransferase (ALT; GPT) (1 case) and γ-GTP (4 cases). These changes were mild and the incidence was almost the same between the losartan group and the amlodipine group. An increase in serum uric acid (2 cases) was reported in the amlodipine group, but was not observed in the losartan group. Hyperkalemia ranging from 5.1 to 6.9 mEq/l was reported in the losartan group (3 cases) and in the amlodipine group (2 cases). Two cases of dizziness and 1 case of transient ischemic attack were reported in the losartan and amlodipine groups. No fatal adverse events were observed in either group during the 12-month study.

Discussion

The present study demonstrated that, in patients with proteinuric CKD and hypertension, losartan effectively reduced proteinuria while amlodipine did not. It is noteworthy that the potency of BP-lowering of losartan and amlodipine was same throughout the entire 12-month study period. Allocation of patients resulted in an almost comparable male to female ratio between the treatment groups at baseline. However, more number of female patients decreased in the amlodipine group than in the losartan group as the study progressed. Consequently, at month 12, in the losartan group, the male/female ratio was 29/18, while in the amlodipine group it was 30/10. Although the losartan group included a greater number of female patients than the amlodipine group at months 3, 6, and 12, the percent reduction in urinary protein excretion in males was comparable to that in females in the losartan group. Therefore, it was unlikely that a sex hormone such as estrogen played a role in the vascular protection in this study. The fact that a large majority of female patients in the losartan group at baseline were aged (22 females: 54–59 year-old, 4; in their 60's, 9; in her 70's, 1) may warrant this discussion, because female patients of mid-50's or older were probably undergoing menopause.

In the present study, we first stratified patients into 3 subgroups with regard to BP reduction measured at month 3. The first 3 months was a meaningful period because no other drugs was added on either losartan or amlodipine during this period. Losartan reduced both BP and proteinuria. However, it was also true that not all patients responded to losartan to reach the goal BP of <130/85 mmHg that was recommended by the JNC-VI (*12*). In fact, the goal BP was achieved in only 8 patients in the losartan group and 13 patients in the amlodipine group. It was expected that patients who reached the goal BP of <130/85 mmHg would show a prominent decrease in urinary protein excretion. However, there was no significant change in urinary protein excretion from baseline in either the losartan group or the amlodipine group, al-

though in the losartan group urinary protein tended to decrease. The reason for this finding is unclear; however, since the number of patients in each group was very small, this might be the reason why we failed to demonstrate statistical significance, especially in the losartan group. Nonetheless, even in patients who did not accomplish the BP goal, reduction of proteinuria was evident. Likewise, patients who achieved a BP of $<140/90$ mmHg represented the anti-proteinuric effect of losartan. A striking evidence was that patients who did not accomplish the level of BP $<140/90$ mmHg also showed the reduction in proteinuria, the degree of which did not largely differ from those in the group of BP $<140/90$ mmHg.

It must not be a conclusion that, in patients with CKD and hypertension, it is sufficient to pursue a reduction in proteinuria without a corresponding reduction in BP. It should be emphasized that BP control is still an important strategy in treating patients with CKD and hypertension, as the JNC-VI recommends. Our results can only be taken to indicate that losartan may still be effective to reduce proteinuria, even if BP can not reach the BP goal of the JNC-VI guidelines (*12*). In this aspect, losartan should be used in clinical practice under the condition of exerting anti-hypertensive effect. The goal BP of $<130/80$ mmHg for patients with CKD which was currently recommended by JNC-VII guideline (*13*) should also be taken into account. Thus, the use of losartan will bring better outcomes for patients with CKD and hypertension with concomitant BP control.

Although we failed to find a difference in anti-proteinuric effect between losartan and amlodipine when patients were stratified with the baseline proteinuria of <1 g/day and ≥ 1 g/day, further stratification with levels of <2 g/day and ≥ 2 g/day clearly demonstrated the anti-proteinuric effect of losartan at all assay points in the group of ≥ 2 g/day. These results suggest that losartan was effective to reduce severe proteinuria of probably glomerular origin. The effect was still observable in the group of <2 g/day at months 3 and 6, but was not statistically significant at month 12, probably due to a wide range of standard deviation from the mean value. Very recently, Tojo *et al.* (*14*) reported that, in streptozotocin-induced diabetic rats, intervention of actions of angiotensin II by either an ACE inhibitor or an angiotensin II antagonist restored albumin reabsorption in the proximal tubules without changing blood glucose *via* restoration of the expression of megalin, a glycoprotein responsible for reabsorption of proteins in the proximal tubules, resulting in the reduction in urinary protein excretion. The authors suggested that expression of megalin is suppressed in the proximal tubules when the kidney is impaired for tubular dysfunction. This evidence may explain, at least in part, our results on the effect of losartan on proteinuria, a part of which may be of tubular origin.

While the RENAAL study (*8*) was conducted in patients with type 2 diabetes, a large majority of the patients enrolled in the present study had chronic glomerulonephritis including cases of immunoglobulin A (IgA) nephropathy. In these patients, losartan effectively reduced urinary protein excretion. Chronic glomerulonephritis involves many factors in its etiology, and the complicated proteinuria is not solely a result of hyperfiltration of glomeruli. Rather, remodeling of the glomerulus must be considered. Since amlodipine did not affect the protein excretion in such patients, the present result is of particular interest in considering the direct actions of angiotensin II on the structure and functions of glomeruli. Patients with diabetic nephropathy in the losartan group and the amlodipine group were 7 and 5 on the day of start and only 5 and 4 patients completed the study, respectively. Because of this limited number of diabetic patients, there was no statistically significant change in urinary protein excretion in either drug treatment group, although the magnitude of the mean reduction of urinary protein ranged from -30% to -50%. We therefore cannot conclude from these results that these drugs have no anti-proteinuric effect in patients with diabetic nephropathy.

With respect to the pharmacotherapy of patients with CKD, the therapeutic benefit of interfering with the actions of angiotensin II has been extensively documented with ACE inhibitors over the last decade. The breakthrough evidence that direct blockade of angiotensin II receptors protects the kidney in patients with type 2 diabetic nephropathy was provided by the RENAAL study (*8*) with losartan, and the IDNT study with irbesartan (*15*).

Recent publications provided evidences that the angiotensin II receptor antagonist candesartan was effective in Japanese patients with type-2 diabetic nephropathy, with a dose as low as 4 mg/day to prevent aggravation of proteinuria (*16*), or reduce urinary protein excretion by combination therapy with amlodipine (*17*), supporting previous evidences on losartan and irbesartan for diabetic nephropathy. The results of our present study provide the additional information useful in clinical practice, that losartan is effective not only for patients with type 2 diabetic nephropathy, but also those with a variety of types of CKD. Nakao *et al.* (*18*) recently studied the effect of combination therapy and monotherapy with losartan and the ACE inhibitor trandolapril in patients with non-diabetic renal disease. They demonstrated that losartan as well as trandolapril effectively lowered urinary protein excretion, although the combination of these two drugs exerted a more favorable effect on proteinuria. Taken together, the antiproteinuric effect of losartan may play a major role in its renoprotective effect.

The therapeutic benefit of losartan for kidney diseases in comparison to other antihypertensive drugs is still not fully explained. As is indicated in the JNC-VI (*12*) and JNC-VII (*13*) guidelines and several clinical reports, aggressive blood pressure control is mostly important. On the other hand, many clinical trials have demonstrated that blood pressure control is not the only factor pertinent for renoprotection; rather, ACE inhibitors and angiotensin II receptor antagonists provide additional benefit in patients with kidney dis-

eases.

The RAS is now well understood to be involved in the pathogenesis of renal impairment independent of its vasoconstrictive actions, inducing disturbance of glomerular and tubular functions. The direct actions of angiotensin II in the kidney include an increase in tubular sodium reabsorption and an influence on glomerular filtration rate (GFR), but morphopathological changes such as accumulation of extracellular matrix and mesangial cell proliferation and hypertrophy (*19*, *20*) are of more importance for pathogenesis of renal impairment. These concepts clearly constitute the theory of usefulness of blocking the actions of angiotensin II in kidney diseases. Although the UK Prospective Diabetes Study (UKPDS) (*21*) concluded that the effects of ACE inhibitor captopril and the β-blocker atenolol were similar in reducing the risk of macrovascular and microvascular complications related to type 2 diabetes, the African-American Study of Kidney Disease and Hypertension (AASK) Study (*22*), which compared the effects of the ACE inhibitor ramipril, the calcium channel blocker amlodipine, and the β-blocker metoprolol on the progression of hypertensive renal disease in African-Americans, showed that ramipril induced a slower decline in GFR and a lower risk of clinical end points compared to amlodipine.

The mechanism and mode of action of losartan and amlodipine to explain the exertion of different effect of renoprotection are not thoroughly explained and are controversial. Documents are available to explain the renoprotective efficacy of calcium channel blockers, including amlodipine. However, whether calcium channel blockers exert unique antiproteinuric effects is still controversial. In the AASK Study (*22*), proteinuria was not decreased with amlodipine. The Japan Multicenter Investigation of Antihypertensive Treatment for Nephropathy in Diabetes (J-MIND) study (*23*) reported that nifedipine retard and enalapril had a similar effect on nephropathy in hypertensive type 2 diabetic Japanese patients, but albumin excretion rate was not reduced with either drug despite the effective BP lowering. Kumagai *et al.* (*24*) reported the comparative evaluation of amlodipine with ACE inhibitors enalapril or captopril for renoprotective effect in hypertensive patients with renal dysfunction. They concluded that the effect of 1-year treatment with amlodipine on renal function was likely the same as that of ACE inhibitors. They also showed that urinary protein excretion tended to be reduced by either ACE inhibitor or amlodipine, but without statistical significance. These evidences suggest that, while a strong argument has been made for proteinuria as a risk factor for progression of renal disease (*25*), there is still a discrepancy between renoprotection as a final goal and urinary protein excretion as an important clinical sign for renal dysfunction.

There is thus a strong body of evidence suggesting that the pathways by which angiotensin II aggravates renal functions are mediated by angiotensin II type 1 (AT_1) receptors. Calcium channel blockers act to dilate the microvasculature, improving regional circulation by regulating the voltage-dependent calcium channels. The blockade of angiotensin II receptors results in a reduction in renal perfusion pressure in addition to dilation of the efferent arterioles to a greater extent than the afferent arterioles because of their different manner of constriction in response to angiotensin II, and thus angiotensin II antagonists reduce the glomerular filtration pressure to same extent. On the other hand, the action of angiotensin II is not solely to constrict macrovascular and microvascular trees, but a variety of cellular actions are evident. A number of reports have described roles of angiotensin II through AT_1 receptors to produce extracellular matrix as well as to stimulate proliferation and/or hypertrophy of many types of cells; *via* the direct stimulation of mitogen-activated protein kinase (MAPK), transforming growth factor (TGF-β), nuclear factor (NF-κB), induction of proto-oncogenes, and so on (*19*, *20*, *26*). Thus, although there is still no confirmatory theory, wider biological functions of angiotensin II may explain the diversity of renoprotective activity of the two drugs without depending on their BP lowering efficacy. The precise mechanism of the action of these drugs should be further investigated.

In the present study, there was no change in Ccr either in the losartan or amlodipine groups. Andersen *et al.* (*27*) conducted a 2-month, randomized, double-blind cross-over clinical trial to evaluate the effect of losartan and the ACE inhibitor enalapril in patients with type 1 diabetic nephropathy, and reported that angiotensin II blockade reduced urinary protein excretion without changing GFR. In the RENAAL study (*8*), the risk of a doubling of the serum creatinine concentration in the losartan treatment group and the placebo group was almost the same until 12 months from initiation of the study, although the reduction in urinary protein excretion was observed in the losartan treatment group within 6 months. The IDNT study (*15*) with irbesartan also reported no difference in the change in serum creatinine in comparison to placebo and amlodipine within 12 months. Thus, it is likely that effects on proteinuria and on Ccr differ in response to blockade of angiotensin II receptors, although the reason is not explained. The present study was completed at 12 months. It might be expected that longer-term treatment of the patients with CKD and hypertension with losartan would have more beneficial effects on renal functions such as improvement of GFR in patients beyond the effect to reduce proteinuria.

In conclusion, a term of total 12 months treatments of Japanese patients with proteinuric CKD and hypertension with losartan reduced proteinuria more effectively than amlodipine, although BP lowering effect was not different between the two drug-treated groups. Since the effect was beyond the blood pressure control, losartan is effective in patients with CKD manifesting proteinuria and hypertension.

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Induction of uPA release in human peripheral blood lymphocytes by [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP)

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[1]*Department of Plastic and Reconstructive Surgery and* [3]*Environmental Biochemistry, Graduate School of Medicine, Chiba University, Chiba 260-8670; and* [2]*Division of Clinical Research, Sakura National Hospital, Sakura 285-8765, Japan*

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Yamaguchi, Yoshitaka, Kenichi Yamada, Toshikazu Suzuki, Yu-Ping Wu, Kazuko Kita, Shunji Takahashi, Masaharu Ichinose, and Nobuo Suzuki. Induction of uPA release in human peripheral blood lymphocytes by [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP). *Am J Physiol Endocrinol Metab* 287: E970–E976, 2004. First published June 15, 2004; doi:10.1152/ajpendo.00027.2003.—[deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP), known to be an arginine vasopressin (AVP) V_2 receptor agonist, is an agent that increases fibrinolytic activity levels in plasma after its infusion into the human body. However, mechanisms underlying an increase and exact localization of the extrarenal dDAVP-responsive V_2 receptor remain unclarified. Two AVP receptors, V_{1a} and V_2, and a related oxytocin (OT) receptor were found to be expressed in human lymphocytes. Furthermore, we found an increase of fibrinolytic activity in the medium of peripheral lymphocytes obtained from human volunteers less than 20 min after dDAVP infusion. The increased activity was also detected in the medium after incubating the lymphocytes in the presence of dDAVP in vitro, being highest at 20 min after the incubation. In accord with the increased fibrinolytic activity, the levels of urokinase-type plasminogen activator (uPA) in the medium were also increased. However, there was no significant difference of plasminogen activator inhibitor-1 (PAI-1), pro-uPA, and tissue-type plasminogen activator (tPA) concentrations in the medium between dDAVP treatment and control. When lymphocytes were preincubated with a V_2 receptor antagonist [Adamantaneacetyl^1,O-Et-D-Tyr^2,Val^4,Aminobutyryl^6,Arg^{8,9}]-vasopressin, the dDAVP-induced uPA increase was diminished. In contrast, preincubation with a V_1 receptor antagonist, [β-Mercapto-β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin, prior to dDAVP treatment resulted in a greater increase of the uPA concentration in the medium than with the dDAVP treatment alone. Thus it was suggested that dDAVP may induce uPA release from human lymphocytes via V_2 receptor-mediated reaction, and also via cross-talk between V_1 and V_2 receptors.

arginine vasopressin; plasminogen activator; urokinase-type plasminogen activator; protease release

PROTEASE ACTIVITY IN HUMAN LYMPHOCYTES is an intriguing topic because of its involvement in various senescence-associated diseases, neural migration, or demyelination disorders (31–33), although all of the proteases involved in these disorders have not been well characterized. To elucidate the role of protease activity, we recently established a method to search for agents that increase the protease activity levels in lymphocytes freshly prepared from human peripheral blood (34). In this new method, fibrinolytic activity is estimated by incubating lymphocytes with ^{125}I-labeled fibrin as a substrate in the presence of plasminogen. This cascade reaction amplifies the activity levels and therefore is useful for detecting protease activation events of stress response in the human body (34). In particular, this reaction assay in vitro will reflect the protease activation induced by drugs in vivo.

Arginine vasopressin (AVP) and oxytocin (OT) are cyclic nonapeptides whose actions are mediated by stimulation of specific G protein-coupled receptors classified into V_{1a} (vascular), V_{1b} (pituitary), and V_2 (renal) receptors and OT receptors (16, 18). All members of the family have been cloned, and the affinity of cloned AVP and/or OT receptors for [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) and other ligands is well described (19, 36). AVP directly elicits the contraction of smooth muscle preparation via V_1 receptor activation. On the other hand, V_2 receptors in renal tubular cells promote the reabsorption of water (4). It has also been reported that, in canine basilar artery, AVP causes an endothelium-dependent relaxation via the V_{1a} receptor (9) and that, in experimental animals (28) or humans (6), 4-valine-8-D-arginine vasopressin or dDAVP causes a decrease in blood pressure that is not mediated by prostaglandins (13, 16). In rat aortic strips, dDAVP evokes endothelium-dependent vasorelaxation (40), not via the authentic V_2 receptor but rather via the endothelial V_1-like receptors, which may be functionally different from the V_1 receptor in smooth muscle cells. OT is another posterior pituitary hormone whose primary action is to stimulate uterus contraction or milk ejection function via OT receptors (35). In myometrium, binding of OT to high-affinity receptors stimulates various biological responses, including inositol-triphosphate turnover and Ca^{2+} influx, similar to those induced by the binding of AVP to the V_{1a} receptors. Interestingly, the uterus contains not only OT receptors but also V_{1a} receptors of approximately fivefold higher density in nonpregnant conditions (2). OT has also been reported to enhance glomerular filtration rate and to have a natriuretic effect (3). In addition, OT has either diuretic or antidiuretic osmoregulatory effects depending on the presence or absence of vasopressin, which may be explained by the ability of OT to bind to the adenylate cyclase-stimulating V_2 receptor in distal tubules and collecting ducts (5, 15). Because some of the organs or cells express different subtypes of AVP/OT receptors, the cross-talk between them may be involved in various unknown physiological events.

dDAVP is known to be an agonist for one of the three types of AVP receptors, namely V_2 (16, 18). It was reported that a marked increase of fibrinolytic activity in plasma is observed when dDAVP is infused in humans (17). This increase paral-

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leled that of immunoreactive tissue-type plasminogen activator (tPA), which is partly derived from vascular endothelial cells (12). It was also demonstrated that dDAVP induces the release of two coagulation factors, factor VIII and von Willebrand factor, from the vascular endothelium through the activation of a vasculoendothelial V_2-like receptor (11, 16, 26). However, the mechanism by which dDAVP causes the increase of fibrinolytic activity in plasma remains unclarified.

Human peripheral lymphocytes possess binding sites for dDAVP (38), although the expression of the receptor for binding dDAVP has not been determined. In addition, neither the role of V_2 receptor in lymphocytes nor the action of V_2 receptor agonist in peripheral blood lymphocytes has been documented until now.

In the present study, we determined the expression of mRNA species of AVP/OT receptors that can bind to dDAVP in human peripheral lymphocytes. Then, we examined whether peripheral lymphocytes obtained from human subjects with dDAVP infusion have the ability to increase plasminogen-dependent fibrinolytic protease activity and whether lymphocytes release the proteases when incubated with dDAVP in vitro.

MATERIALS AND METHODS

Agents. dDAVP and AVP were purchased from Ferring Pharmaceuticals (Copenhagen, Denmark). [β-Mercapto-β,β-cyclopentamethylenepropionyl[1],O-Me-Tyr[2],Arg[8]]-vasopressin, [Adamantaneacetyl[1],O-Et-D-Tyr[2],Val[4],Aminobutyryl[6],Arg[8,9]]-vasopressin, and fibrinogen were obtained from Sigma Chemical (St. Louis, MO). [^{125}I]Na (100 mCi/ml, 17 mCi/mg) was purchased from New England Nuclear (Boston, MA).

Infusion study. dDAVP was diluted with saline to allow for the intravenous delivery of 0.4 μg/kg in 100 ml over 10 min (6). Two milliliters of whole blood samples were withdrawn from cubital veins of 12 volunteers (6 males and 6 females from 40 to 60 yr old, mean age 47.3), with EDTA as an anticoagulant, every 10 min after dDAVP infusion. As a control, samples were taken from 12 age-matched volunteers (6 males and 6 females) without dDAVP infusion. Informed consent was obtained from all of the volunteers, and this study was approved by the Human Research Committee of Sakura National Hospital.

Preparation of lymphocyte samples. Lymphocytes were prepared from peripheral blood of volunteers principally according to the method described previously (32). Briefly, each (2-ml) blood sample was diluted with the same volume of phosphate-buffered saline [PBS; 10 mM sodium phosphate (pH 7.4) containing 135 mM NaCl] and put on 7 ml of Ficoll. The samples were centrifuged at 240 *g* for 20 min at room temperature. After centrifugation, the thin white layer of the lymphocyte fraction, termed "buffy coat," was collected and mixed with a fivefold volume of PBS. Then, it was centrifuged at 240 *g* for 10 min. The pellet was suspended in RPMI 1640 medium and incubated in a 60-mm dish for 20 min at 37°C for platelet attachment. Flow cytometric analysis (FACS) with anti-CD2 antibodies proved that the lymphocyte samples we used in this study contained ~95% T cells (39). Contaminations by B cells and monocytes were negligible in this separation procedure by FACS that used anti-CD13, -CD14, -CD16, and -CD17 antibodies. The lymphocyte samples were diluted with RPMI 1640 medium to make solutions containing appropriate numbers of cells (10^4 to 10^6 cells/ml).

RNA isolation and RT-PCR. Total RNA was isolated from lymphocyte samples, MCF-7 human breast cancer cells, and human umbilical vein endothelial cells (HUVEC) by use of TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. MCF-7 cells and HUVEC were used as positive controls for expression of the three AVP receptors (V_{1a}, V_{1b}, and V_2) and the OT receptor, respectively (23, 37). After treatment with deoxyribonuclease I (Invitrogen) to eliminate possible DNA contamination, the first-strand cDNA synthesis was carried out by use of SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) with 2 μg of total RNA and 0.5 μg of oligo(dT). Thereafter, a 1-μl aliquot of the first-strand cDNA was used together with 200 nM of each specific primer, PCR buffer (in mM: 10 Tris·HCl, pH 8.3, 50 KCl, and 1.5 $MgCl_2$), and 1 unit of recombinant *Taq* DNA polymerase (TaKaRa, Kyoto, Japan) to a total volume of 25 μl. The PCR primer sequences for AVP/OT receptors used in this study were exercised according to a previous report by Thibonnier et al. (37), and glyceraldehyde 3-phosphate dehydrogenase was used as a positive control for each RNA preparation. The amplification was performed in a TaKaRa thermal cycler (model TP-400) with the following steps: initial denaturation at 95°C for 5 min, followed by 35 cycles: 95°C for 30 s, 56°C for 1 min, 72°C for 1 min, and an additional extension at 72°C for 5 min. The PCR products were visualized after electrophoresis in a 2.0% agarose gel with ethidium bromide staining.

In vitro incubation of lymphocytes with or without drugs. Lymphocyte samples were incubated in RPMI 1640 medium with various concentrations of dDAVP or AVP for 20 min at 37°C in vitro. After the incubation, supernatants were obtained by centrifugation of samples for 5 min at 300 *g* and used for further analysis.

Preincubation of lymphocyte samples (10^6 cells/ml) with receptor antagonists was performed for 20 min at 37°C. After the preincubation, samples were further incubated with or without dDAVP (10^{-8} M) for another 20 min at 37°C and then centrifuged at 300 *g* for 5 min. The supernatant was used for the protease assay.

Assay of fibrinolytic activity. Fibrinogen was labeled with [^{125}I]Na by the chloramine-T method (7) and then used for preparing polystyrene tubes coated with ^{125}I-labeled fibrin (29). ^{125}I-labeled fibrinogen had a specific radioactivity of 1.0 mCi/mg protein.

The lymphocyte samples and RPMI 1640 medium without lymphocytes (as a control) were incubated in ^{125}I-fibrin-coated tubes at 37°C for 20 min in the presence of plasminogen. The released radioactivity (counts/min or cpm) of ^{125}I was counted as described previously (29). The radioactivity increased linearly during the incubation for 1 h.

Measurement of tPA, urokinase-type plasminogen activator, and plasminogen activator inhibitor-1 concentrations. The concentrations of tPA, pro-urokinase-type plasminogen activator (pro-uPA), and plasminogen activator inhibitor (PAI)-1 in the supernatant of the samples after in vitro incubation of lymphocytes were measured using the corresponding assay kits, Chromolize tPA Assay Kit, Chromolize uPA Assay Kit, and Imulyse PAI-1, respectively (all from Biopool International, Ventura, CA). The uPA assay was performed using AngioMax Human Urokinase (uPA) ELISA Kit (Angiopharm, O'Fallon, MO). The reaction was carried out at room temperature throughout the assay.

Statistical analysis. Values are presented as means ± SD. Statistical analysis was performed by Student's *t*-test with StatView software (SAS Institute, Cary, NC).

RESULTS

Expression of AVP/OT receptors in human lymphocytes. In the beginning of the study, the mRNA expression of specific AVP/OT receptors in human lymphocytes from peripheral blood was determined by RT-PCR analysis (Fig. 1). Expression of V_{1a}, V_2, and OT receptors was observed in all lymphocytes of four independent donors. On the other hand, we could not detect the expression of the V_{1b} receptor either in human lymphocytes or in MCF-7 cells (data not shown). Two amplified DNA bands were detected from lymphocytes as well as from MCF-7 cells when we analyzed the expression of V_2

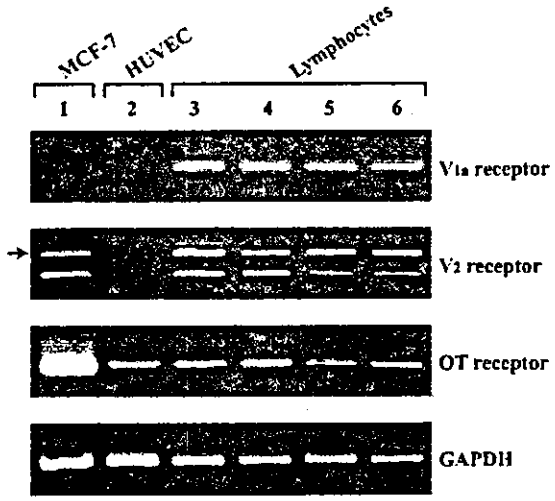

Fig. 1. Expression of arginine vasopressin (AVP) V_{1a} and V_2 and oxytocin (OT) receptors in human lymphocytes. MCF-7 cells (*lane 1*) and human umbilical vein endothelial cells (HUVEC, *lane 2*) were used as positive controls for expression of AVP/OT receptor mRNAs. *Lanes 3–6*, human lymphocytes from 4 independent donors. Arrow, amplified DNA corresponding to an alternative form of V_2 receptor mRNA. GAPDH, positive control for each RNA preparation.

receptor mRNA (Fig. 1). According to a previous report (23), MCF-7 cells express an alternative form of V_2 receptor containing the entire 106 bases of intron 2 in addition to a sequence for V_2 receptor mRNA as well as normal forms. Therefore, it was suggested that both normal and alternative forms of V_2 receptor mRNA are expressed in human lymphocytes.

Effect of dDAVP infusion on fibrinolytic activity. Each lymphocyte sample was prepared up to 40 min after infusion in humans with or without dDAVP. The levels of fibrinolytic activity in the medium of incubated lymphocytes were significantly increased and reached the peak at 20 min after the infusion, followed by a decrease to the basal level (Fig. 2).

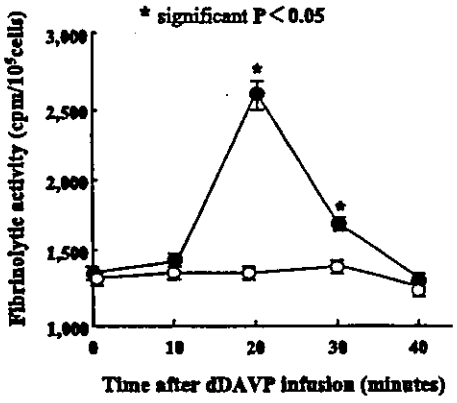

Fig. 2. Fibrinolytic activity in the medium of incubated peripheral blood lymphocytes after [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) infusion (●) and without infusion (○). Values represent the average ± SD of 3 independent experiments.

Effect of dDAVP or AVP treatment on fibrinolytic activity in vitro. To investigate the kinds of proteases released in the medium, lymphocyte samples were incubated with dDAVP or AVP. The levels of fibrinolytic activity in the medium were highest at 10^{-8} M of dDAVP, and the dose-response curve appeared to be bell-shaped (Fig. 3*A*). However, AVP treatment did not result in the increased levels of fibrinolytic activity at any dose examined (Fig. 3*B*).

Effect of dDAVP on tPA, pro-uPA, and uPA concentrations. We next measured the concentrations of tPA, pro-uPA, and uPA in the medium of lymphocyte samples incubated with dDAVP ($\leq 10^{-6}$ M). There were no significant differences in the tPA and pro-uPA concentrations between dDAVP treatment and control (Fig. 4, *A* and *B*). However, a significant increase in the levels of uPA was observed after incubating lymphocytes with dDAVP, showing a bell-shaped pattern with the highest level at 10^{-8} M dDAVP (Fig. 4*C*).

Effect of dDAVP on PAI-1 concentration. The uPA assay kit used in this study recognizes both PAI-1-free (active) and PAI-1-bound (inactive) uPA. To examine whether uPA was detected as an active form or as an inactive PAI-1-bound form, we measured levels of PAI-1 in the medium after incubating lymphocytes with dDAVP (10^{-8} M). No increase was observed in total levels of PAI-1 concentration up to 120 min

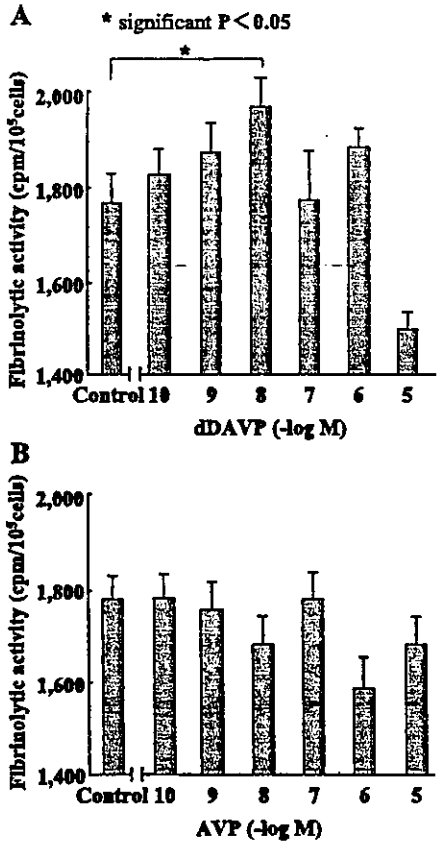

Fig. 3. Fibrinolytic activity in the medium after incubation of lymphocytes with dDAVP (*A*) and AVP (*B*). Values represent the average ± SD of 3 independent experiments.