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Effect of telmisartan on vascular endothelium in hypertensive and type 2 diabetic hypertensive patients

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Aim: Hypertension and type 2 diabetes mellitus (DM) cause endothelial dysfunction and may result in cardiovascular disease. The aim of this study was to assess endothelial dysfunction in essential hypertensives, and normotensive and hypertensive type 2 diabetics and to evaluate the effect of telmisartan on endothelium in hypertensives.

Materials and methods: Eighteen essential hypertensives (group 1), 16 type 2 diabetic hypertensives (group 2), 10 type 2 diabetic normotensives (group 3), and 10 control subjects (group 4) were included in this study. Groups 1 and 2 received 40 mg/day telmisartan for 12 weeks and were evaluated at the beginning and end. Groups 3 and 4 were evaluated once by serum nitrate (NO), vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), thrombomodulin (TM), plasminogen activator inhibitor-1 (PAI-1), paraoxonase (PON1), urine microalbumin (MAU), and endothelium dependent flow mediated dilation (FMD).

Results: In groups 1, 2, and 3, PAI-1 ($P < 0.001$, for all) and MAU ($P = 0.012$, $P = 0.006$, $P = 0.004$, respectively) were significantly higher than they were in group 4. In group 2, PON1 was significantly lower than it was in groups 4 and 1 ($P = 0.028$, $P < 0.001$ respectively), and NO was significantly lower than it was in groups 1, 3, and 4 ($P < 0.001$, for all). Brachial artery FMD was significantly lower in groups 1 and 2 than it was in group 4 and FMD in group 2 was lower than it was in group 3. After telmisartan treatment there were significant increases in PON1 in groups 1 and 2, and in TM in group 2.

Conclusion: Type 2 DM and essential hypertension result in endothelial dysfunction. Telmisartan decreases blood pressure to normal ranges in hypertensives, but it has a minimal role in improvement of endothelial dysfunction.

Key words: Endothelial dysfunction, essential hypertension, type 2 diabetes mellitus

Hipertansif ve tip 2 diabetik hipertansif olgularda telmisartanin damar endoteli üzerine etkisi

Amaç: Hipertansiyon ve tip 2 diabetes mellitus (DM) endotel disfonksiyonuna neden olup kardiyovasküler hastalık ile sonuçlanabilir. Bu çalışmada amaç; esansiyel hipertansif, normotansif ve hipertansif tip 2 diabetik olgularda endotel disfonksiyonunu ve hipertansiflerde telmisartanın endotel üzerine etkisini belirlemekti.

Yöntem ve gereç: Çalışmaya 18 esansiyel hipertansif (grup 1), 16 tip 2 diabetic hipertansif (grup2), 10 tip 2 diabetik normotansif (grup 3) ve 10 kontrol (grup 4) dahil edildi. Grup 1 ve 2'ye 12 hafta boyunca 40 mg/gün telmisartan uygulandı ve başlangıçta ve tedavi sonunda, grup 3 ve 4 ise sadece bir defa olmak üzere serum nitrat (NO), vaskuler hücre adhezyon molekülü-1 (VCAM-1), trombosit endothel hücre adezyon molekülü-1 (PECAM-1), trombomodulin (TM), plazminojen aktivator inhibitörü-1 (PAI-1), paraoxonaz (PON1), idrar mikroalbumini (MAU) ve akıma bağlı dilatasyonla (ABD) parametreleri aracılığı ile değerlendirildi.

Bulgular: Grup 1, 2 ve 3'te PAI-1 (tümü, $P < 0,001$) ve MAU (sırayla, $P = 0,012$, $P = 0,006$, $P = 0,004$) grup 4'e göre belirgin olarak yüksekti. Grup 2'de; PON1, grup 4 ve 1'e göre (sırayla, $P = 0,028$, $P < 0,001$), NO grup 1, 3, 4'e göre (tümü, $P < 0,001$) anlamlı olarak düşük bulundu. Brakiyal arter akıma bağlı dilatasyon grup 4'e göre grup 1 ve 2'de, ayrıca grup

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3'e göre grup 2'de anlamlı olarak düşüktü. Telmisartan tedavisinden sonra kan basıncı anlamlı olarak düşmekte olup, grup 1 ve 2'de PON1 ve grup 2'de TM düzeyleri anlamlı olarak yüksek bulundu.

Sonuç: Tip 2 DM ve esansiyel hipertansiyona endotelial disfonksiyonu eşlik etmektedir. Telmisartan hipertansiflerde kan basıncını normal değerlere çekmekte, fakat endotel disfonksiyonu üzerine minimum düzeltici etki oluşturmaktadır.

Anahtar sözcükler: endotel disfonksiyonu, esansiyel hipertansiyon, tip 2 diabetes mellitus

Introduction

Endothelial dysfunction, an early event in the development of atherosclerosis, is characterized by a reduced endothelium-dependent vasodilation and might have prognostic value for future cardiovascular events. Traditional risk factors like hypercholesterolemia, hypertension, cigarette smoking, and diabetes mellitus are associated with endothelial dysfunction, which is reliably shown by efficacious plasma biomarkers and peripheral vascular imaging analysis (1,2).

The renin-angiotensin system (RAS) is an important component of blood pressure regulation. Angiotensin II, the most important active factor in the RAS, has several functions, including stimulation and inhibition of cell proliferation, generation of reactive oxygen species (ROS), induction of apoptosis, and regulation of proinflammatory and profibrogenic actions (3). Telmisartan is an angiotensin II antagonist and it blockades angiotensin II receptor and is used for antihypertensive therapy (4). Some classes of antihypertensives such as calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II receptor blockers (ARBs) have been shown to reduce the incidence of new onset diabetes, especially compared to diuretics and β-blockers (5). Telmisartan, unlike other ARBs, acts as a partial peroxisome proliferator-activated receptor gamma (PPARγ) agonist (6).

The aims of the present study were to evaluate endothelial dysfunction in essential hypertensive, normotensive, and hypertensive type 2 diabetic patients and the effects of telmisartan on endothelium in hypertensive patients by serum nitrate (NO), vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), thrombomodulin (TM), plasminogen activator inhibitor-1 (PAI-1), paraoxonase (PON1), urine microalbumin (MAU), and endothelium dependent flow mediated dilation (FMD).

Materials and methods

Patients

Patients with essential hypertension (group 1), type 2 diabetic hypertension (group 2), and type 2 diabetic normotension (group 3) had been diagnosed at the departments of Cardiology and Endocrinology of Ege University Hospital, and were randomly selected from the out-patient clinic population. Normal control subjects (group 4) were selected from among healthy hospital stuff. All subjects were informed of the investigational nature of the study and agreed to participate. Informed consent was obtained from all participants. The protocol was approved by the local Ethical Committee in accordance with the Declaration of Helsinki.

Diagnostic criteria for essential hypertension and diabetes mellitus

For essential hypertension, subjects with systolic blood pressure 140-180 mmHg and/or diastolic blood pressure 90-110 mmHg on 2 consecutive follow-up visits were recruited.

For type 2 diabetes, subjects with fasting serum glucose concentration over 126 mg/dL on 2 consecutive analyses, postprandial serum glucose over 200 mg/dL, or serum glucose level at 2 h of more than 200 mg/dL in the oral glucose tolerance test were recruited.

Secondary hypertension was excluded. None of the subjects were taking antihypertensive, anti-inflammatory, or antilipidemic drugs, nitrates, vitamins, or other known medication influencing endothelial function, and none had a history of cardiovascular disease, type 1 diabetes mellitus, peripheral vascular disease, renal disease, or other inflammatory or non-inflammatory systemic disease. Type 2 diabetic patients received their routine oral antidiabetic treatment throughout the study period. None of the control subjects were taking any medication.

Clinical Protocol

Groups 1 and 2 received 40 mg/day telmisartan for 12 weeks and were evaluated at the beginning and at the end. Groups 3 and 4 were evaluated once via laboratory and vascular analyses.

Laboratory Analysis

After 12-h overnight fasting blood samples were obtained and the first urine sample in the morning was collected. Lipid, glucose, creatinine, uric acid, hemoglobin A1c (HbA1c), high sensitive C-reactive protein (hsCRP), and urine albumin measurements were performed on the same day. Serum samples for sVCAM, sPECAM, PAI-1, TM, NO, and PON1 were stored at –80 °C prior to analysis.

Serum concentrations of sVCAM-1 (Bender MedSystems, Vienna, Austria), sPECAM-1 (Bender MedSystems, Vienna, Austria), PAI-1 (Tecnoclone®, Vienna, Austria), and TM (Euroclone®, UK) were determined in duplicate by ELISA system (Sanofi Pasteur PR 2100, France). Serum NO concentration was measured by enzymatic endpoint assay with nitrate reductase derived from aspergillus on a Hitachi 902 automatic analyzer (Roche Diagnostics, Hitachi, Tokyo, Japan). Nitrite was produced directly by nitrate reductase and the decrease in absorbance depending on NADPH oxidation was measured at 340 nm (7). Serum PON1 activity was measured by kinetic spectrophotometric method. The rate of hydrolysis of paraoxon to paranitrophenol and diethylphosphoric acid by paraoxonase was determined at 405 nm (8). MAU (Dialab, Vienna, Austria) was measured by immunoturbidimetric method. A Hitachi 704 automatic analyzer (Roche Diagnostics, Hitachi, Tokyo, Japan) was used for assays of PON1 and MAU levels.

Total cholesterol, triglycerides, HDL-cholesterol, glucose, urea, creatinine, and uric acid levels were measured with routine methods on an automatic analyzer (Tecnicon Dax 48, Bayer Diagnostics, Toshiba, Tokyo, Japan). Hemoglobin A1c (HbA1c) and high sensitive C-reactive protein (hsCRP) were measured immunologically on a Hitachi 704 automatic analyzer (Roche Diagnostics, Hitachi, Tokyo, Japan). Calculation of the HbA1c concentration as a percentage was performed according to the DCCT/NGSP correction formula. LDL-cholesterol was calculated by Friedewald's formula.

Vascular analysis

The noninvasive determination of endothelial dysfunction was performed according to Celermajer's method (9). Brachial artery imaging was performed by high-resolution ultrasound with a 7.5-MHz linear array transducer (Hewlett Packard 4500, Hewlett Packard Co. Andover, MA, USA). All subjects were studied in the morning having abstained from alcohol, caffeine, and food for 8 h before the observation. The subjects remained at rest in the supine position for at least 15 min. Each subject's right arm was immobilized in extended position to allow consistent recording of the brachial artery 2-4 cm above the antecubital fossa. Recordings of B-mode and pulsed doppler spectral curve were measured at rest (baseline), during reactive hyperemia (endothelium-dependent vasodilation), and after sublingual isosorbide dinitrate (endothelium-independent vasodilation) administration. Baseline measurements included brachial artery diameter and flow velocity. Reactive hyperemia was created by inflating the cuff to 240 mmHg for 4.5 min on the upper arm. Flow velocity was measured within 15 s of cuff deflation. Blood flow, pressure, and end-diastolic diameter were recorded at 30 s intervals for 5 min and at 6, 8, and 10 min until recovery to baseline values. After 15-20 min (at baseline conditions) measurements of arterial diameter and flow velocity were repeated, followed by sublingual 5 mg isosorbide dinitrate administration to assess endothelium-independent vasodilation. After 4 min arterial diameter and flow velocity were measured. Arterial diameter is the distance measured in millimeters between the anterior wall media adventitia interface (M-mode) and posterior wall intima-lunimal interface at end-diastole. The maximum FMD and NMD arterial diameters were calculated as the average of the 3 consecutive maximum diameter measurements after hyperemia and isosorbide dinitrate, respectively. FMD% and NMD% were then calculated as the diameter change compared to baseline resting diameters.

Statistical analysis

Analyses were performed with the Statistical Package for the Social Sciences (SPSS, version 10.0, SPSS Inc., Chicago, IL, USA) for Windows. In the 4

groups none of the measured parameters showed Gaussian distribution and so nonparametric statistics were applied. All the data were given in median ± quartiles. The 4 independent groups were first compared using the Kruskal-Wallis test and the Mann-Whitney U test was used for comparison between 2 independent groups for significant parameters. Changes in baseline values after telmisartan treatment were compared using the Wilcoxon signed ranks test. Spearman-Rho nonparametric correlation coefficient was used to assess the relation between variables. Multiple stepwise regression analysis was performed to assess the effects of BMI, age, and smoking as independent variables on sVCAM, sPECAM, PAI-1, TM, NO, and PON1. Two-tailed P values <0.050 were considered statistically significant.

Results

Table 1 shows the demographic characteristics and basal values of all groups. In Table 2 values of blood pressure, FMD, NMD, and biochemical parameters before and after telmisartan therapy of essential and

Table 1. Demographic characteristics and baseline values of the 4 groups (values are expressed as median and 1st-3rd quartile).

	Group 1	Group 2	Group 3	Group 4
Age (years)	52 (45-56)	52 (50-58)	53 (46-59)$^{\$}$,**	43 (40-46)
Sex (Male/Female)	12/6	7/9	7/3	6/4
BMI (kg/m²)	28.1 (25.9-29.9)**	27.4 (23.6-30.1)**	26.3 (25.1-27.3) *	21.8 (19.7-25.4)
Smokers (%)	5.55	12.5$^{\$}$	10	10
Creatinine (μmol/L)	70.0 (52.2-79.6)	59.2 (49.5-73.4)	63.6 (49.5-84.9)	70.0 (49.5-70.7)
Uric acid (mmol/L)	0.28 (0.22-0.35)	0.28 (0.24-0.35)	0.27 (0.23-0.31)	0.27 (0.22-0.30)
SBP (mmHg)	150.0 (140.0-155.0) ***	157.5 (150.0-160.0) ***,$^{\dagger\dagger,\$}$	110.0 (98.8-116.3)	110.0 (106.3-121.3)
DBP (mmHg)	75.0 (72.5-82.5) *	80.0 (73.25-84.0) **,†	70.0 (65.0-78.8)	70.0 (67.5-75.0)
FMD (%)	9.38 (3.60-15.24) **	6.41 (5.18-11.20) ***,†	17.32 (12.45-19.14)	20.15 (16.79-23.63)
NMD (%)	13.77 (11.96-23.07)	10.66 (8.09-15.06)	10.58 (9.86-14.18)	16.30 (11.59-25.85)
NO (μmol/L)	40.10 (19.85-51.28)	10.0 (1.7-12.6) ***,$^{\dagger\dagger,\ \$\$\$}$	27.55 (24.53-34.35)	41.50 (31.93-55.80)
PON1 (U/L)	161.0 (139.5-218.5)	139.0 (122-154.3) ***,$^{\$}$	155.0 (138.6-164.5)	169.0 (157.3-179.8)
PAI-1 (μg/L)	199.6 (151.4-257.2) ***	136.6 (102.6-195.7) ***	108.9(87.7-170.1) ***	42.6 (15.6-72.4)
TM (μg/L)	4.00 (3.30-4.78)	4.08 (3.38-5.42)	4.45 (4.02-4.64)	4.45 (2.98-5.16)
PECAM-1 (μg/L)	54.83 (47.32-60.75)	47.51 (41,16-54,09)	46.92 (37.91-52.46)	42.63 (37.22-52.92)
VCAM-1 (μg/L)	825.0 (583.0-1211.0)	747.0 (460.2-975.3)	962.1 (887.2-1094.4)	419.4 (176.3-1236.8)
MAU (g/mol creatinine)	0.75 (0.45-1.89) *	1.10 (0.60-2.69) **	0.76 (0.61-0.86) **	0.40 (0.24-0.62)
hsCRP (mg/L)	2.52 (0.96-4.47)	2.99 (2.07-3.37)	6.16 (1.19-9.40)	1.93 (1.19-2.14)
T. cholesterol (mmol/L)	5.27 (4.48-6.03) *	5.70 (4.92-6.27) **	5.70 (4.45-6.48) *	4.44 (4.09-4.79)
HDL cholesterol (mmol/L)	1.28 (1.11-1.55)	1.37 (1.09-1.53)	1.06 (1.02-1.11)	1.14 (0.96-1.35)
LDL cholesterol (mmol/L)	3.16 (2.75-3.83) *	3.57 (3.03-3.96) **	3.34 (2.25-4.27)	2.69 (2.36-3.08)
Triglycerides (mmol/L)	1.49 (0.89-2.26) *	1.54 (1.06-2.00) **	2.23 (1.38-2.89) *	0.92 (0.78-1.21)
Fasting glucose (mmol/L)	4.58 (4.27-5.38)	6.44 (5.55-6.72) **,$^{\$\$}$	6.13 (5.72-6.72) *	4.91 (4.22-5.27)
HbA1c (%)	5.7 (5.2-6.1)	6.3 (6.0-6.9) **,$^{\$}$	6.1 (5.9-6.6) *	5.6 (5.3-5.7)

Group 1: Essential hypertensive patients, Group 2: Type 2 diabetic hypertensive patients, Group 3: Type 2 diabetic normotensive patients, Group 4: Normal control subjects. BMI: body mass index. SBP: systolic blood pressure, DBP: diastolic blood pressure, FMD: flow mediated dilation, NMD: nitroglycerine mediated dilation, NO: nitrate, PON1: paraoxonase, PAI-1: plasminogen activator inhibitor-1, TM: thrombomodulin, PECAM-1: platelet endothelial cell adhesion molecule-1, VCAM-1: vascular cell adhesion molecule-1, MAU: microalbuminuria, hsCRP: high sensitive C-reactive protein, HbA1c: Hemoglobin A1c

Compared to group 4; *: P < 0.05, **: P < 0.01, ***: P < 0.001

Compared to group 3; †: P < 0.05, ††: P < 0.001

Compared to group 1; §: P < 0.05, §§: P < 0.01, §§§: P < 0.001

Table 2. Values of vascular and biochemical parameters before and after telmisartan therapy (values are expressed as median and 1st-3rd quartile).

	Group 1		Group 2	
	Before telmisartan	After telmisartan	Before telmisartan	After telmisartan
SBP (mmHg)	150.0 (140.0-155.0)	125.0 (120.0-130.0) ***	157.5 (150.0-160.0)	130.0 (120.0-135.0) **
DBP (mmHg)	75.0 (72.5-82.5)	74.0 (68.0-83.0)	80.0 (73.25-84.0)	71.0 (67.0-77.0) *
FMD (%)	9.38 (3.60-15.24)	10.13 (7.26-17.55)	6.41 (5.18-11.20)	8.04 (6.81-8.78)
NMD (%)	13.77 (11.96-23.07)	14.77 (9.21-21.27))	10.66 (8.09-15.06)	16.01 (13.08-25.61) *
NO (µmol/L)	40.10 (19.85-51.28)	29.60 (21.46-40.03)	10.0 (1.7-12.6)	10.77 (5.11-23.30)
PON1 (U/L)	161.0 (139.5-218.5)	199.5 (175.3-256) *	139.0 (122-154.3)	169.5 (143.3-193.5) *
PAI-1 (µg/L)	199.6 (151.4-257.2)	218.6 (177.3-297.5)	136.6 (102.6-195.7)	119.2 (82.9-183.4)
TM (µg/L)	4.00 (3.30-4.78)	4.04 (3.40-5.24)	4.08 (3.38-5.42)	4.72 (4.25-5.83) *
PECAM-1(µg/L)	54.83 (47.32-60.75)	55.92 (48.30-61.11)	47.51 (41,16-54,09)	46.92 (40.68-50.38)
VCAM-1 (µg/L)	825.0 (583.0-1211.0)	794.1 (719.2-1123.9)	747.0 (460.2-975.3)	766.4 (587.0-1006.2)
MAU (g/mol creatinine)	0.75 (0.45-1.89)	0.48 (0.34-0.82)	1.10 (0.60-2.69)	1.12 (0.72-2.96)
hsCRP (mg/L)	2.52 (0.96-4.47)	1.81 (1.20-3.12)	2.99 (2.07-3.37)	1.85 (1.34-4.12)
T. cholesterol (mmol/L)	5.27 (4.48-6.03)	5.31 (4.77-5.96)	5.70 (4.92-6.27)	5.39 (4.90-6.22)
HDL cholesterol (mmol/L)	1.28 (1.11-1.55)	1.32 (1.11-1.63)	1.37 (1.09-1.53)	1.27 (1.19-1.53)
LDL cholesterol (mmol/L)	3.16 (2.75-3.83)	3.28 (2.64-3.91)	3.57 (3.03-3.96)	3.32 (2.72-4.20)
Triglycerides (mmol/L)	1.49 (0.89-2.26)	1.47 (1.05-2.43)	1.54 (1.06-2.00)	1.99 (1.55-2.27)
Fasting glucose (mmol/L)	4.58 (4.27-5.38)	5.08 (4.63-5.33)	6.44 (5.55-6.72)	6.27 (5.49-7.16)
HbA1c (%)	5.7 (5.2-6.1)	5.7 (5.5-5.9)	6.3 (6.0-6.9)	6,5 (5.9-6.8)

Group 1: Essential hypertensive patients, Group 2: Type 2 diabetic hypertensive patients
SBP: systolic blood pressure, DBP: diastolic blood pressure, FMD: flow mediated dilation, NMD: nitroglycerine mediated dilation, NO: nitrate, PON1: paraoxonase, PAI-1: plasminogen activator inhibitor-1, TM: thrombomodulin, PECAM-1: platelet endothelial cell adhesion molecule-1, VCAM-1: vascular cell adhesion molecule-1, MAU: microalbuminuria, hsCRP: high sensitive C-reactive protein, HbA1c: Hemoglobin A1c
Compared to before treatment; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

type 2 diabetic hypertensive groups are given. NO levels of diabetic hypertensive patients were significantly lower than those of the other groups ($P < 0.001$, for all). MAU levels of essential hypertensive, diabetic hypertensive, and diabetic normotensive patients were significantly higher than those of the normal control group ($P = 0.012$, $P = 0.006$, $P = 0.004$, respectively). Serum PON1 levels of diabetic hypertensive patients were significantly lower than those of essential hypertensive patients and normal control subjects ($P = 0.028$, $P < 0.001$ respectively). PAI-1 levels of essential hypertensive, diabetic hypertensive, and diabetic normotensive patients were significantly higher than those of normal control subjects ($P < 0.001$, for all). Total cholesterol and triglyceride levels of essential hypertensive, diabetic hypertensive, and diabetic normotensive patients were significantly higher than those of normal control subjects ($P < 0.05$, $P < 0.01$, $P < 0.05$, respectively), and LDL-cholesterol levels of essential hypertensive and diabetic hypertensive patients were significantly higher than those of normal control subjects ($P < 0.05$, $P < 0.01$, respectively). TM, PECAM-1, VCAM-1, and HDL-c concentrations of the 4 groups were not significantly different. Brachial artery FMD was significantly lower in hypertensive and diabetic hypertensive patients than healthy subjects ($P < 0.01$, $P < 0.003$ respectively) and also FMD levels of diabetic hypertensive patients were lower than those of diabetic normotensive patients ($P < 0.011$). NMD did not differ significantly between the 4 groups.

There was no statistically significant correlation between variables (data not shown).

Multiple stepwise regression analysis was performed and there were no statistically significant effects of BMI, age, or smoking as independent variables on sVCAM, sPECAM, PAI-1, TM, NO, PON1, or FMD (data not shown).

Blood pressure was regulated in both essential hypertensive and diabetic hypertensive patients by 12 weeks of telmisartan treatment. PON1 levels of essential hypertensive and diabetic hypertensive patients ($P = 0.019$, $P = 0.012$, respectively) and TM and NMD values of diabetic hypertensive patients ($P = 0.034$, $P = 0.028$ respectively) increased significantly, but the other biochemical parameters' levels and FMD did not differ significantly between before and after telmisartan.

Discussion

In this study, 12 weeks of antihypertensive treatment with 40 mg/day telmisartan both in hypertensive and in diabetic hypertensive groups resulted in a significant decrease in systolic blood pressure. Additionally, in the diabetic hypertensive group diastolic blood pressure was reduced significantly. The most important finding of our study is that 40 mg/day telmisartan decreased PON1 levels in both hypertensive and type 2 diabetic hypertensive patients.

PON1 anti-atherogenic molecule is thought to play a role in the favorable vascular effects of high-density lipoproteins, mainly through a reduction in low-density lipoprotein oxidation. PON1 is considered to decelerate the atherosclerotic process, since the protective role of HDL is partly attributed to the impedance of lipid peroxide accumulation (10). In our study PON1 levels of the hypertensive group were not significantly lower than those of the control group, similar to studies carried out by Arca et al. (11) and Rice et al. (12). However, the type 2 diabetic hypertensive group had significantly lower PON1 levels than did the healthy control group and hypertensive group, as was found in studies by Abbott et al. (13) and Gowri et al. (14). They found that PON1 levels of diabetic patients were significantly lower than those of non-diabetic patients. On the other hand, in our study the type 2 diabetic group's PON1 activity was insignificantly lower than that of the control group, similar to the study by Rahmani et al. (15). In our study low dose telmisartan treatment increased PON1 activity in essential hypertensive and diabetic hypertensive patients. Telmisartan may be related to increased PON1 activity and may contribute to the favorable effect on endothelial dysfunction. The serum t.cholesterol, trygliceride, HDL-c, and LDL-c concentrations were not significantly different between before and after telmisartan treatment, which indicated that increasing PON1 is independent of lipid concentrations. The low-dose of other angiotensin II receptor antagonist valsartan treatment did not show a similar effect in type 2 diabetic subjects with hypertension in the study by Saisho et al. (16).

In our study NO levels of the diabetic hypertensive group were significantly lower than those of the essential hypertensive, type 2 diabetic normotensive, and control groups; thus we concluded that hypertension and type 2 DM had an additive negative effect on vascular endothelium and decreased the bioavailability of NO more prominently. In the pathogenesis of vascular complications of diabetes mellitus, an increase in oxidant stress arises as a result of several mechanisms such as diminished expression/activity of endothelial NO synthase and generation of NO, overproduction of reactive oxygen species, and impaired expression/activity of superoxide dismutase in the vascular endothelium (17). In our study brachial artery FMD was significantly lower in essential hypertensive and diabetic hypertensive patients than it was in healthy subjects and FMD of diabetic hypertensive patients was lower than that of diabetic normotensive patients. Ghiadoni et al. reported that 80-160 mg/day telmisartan for 6 months did not result in significantly increased FMD in essential hypertensive groups (18). Administration of low dose AT1-receptor antagonist telmisartan did not improve NO or FMD in our study population of hypertensive patients with and without type 2 diabetes mellitus and this indicates that telmisartan may affect vascular tonus by other pathways such as prostacyclin or endothelium derived hyperpolarizing factor.

In several studies a positive correlation has been shown between microalbuminuria and blood pressure (19,20). In our study MAU levels of the hypertensive,

diabetic hypertensive, and diabetic normotensive groups were significantly higher than those of the control group. The diabetic hypertensive group did not differ significantly from the essential hypertensive and diabetic normotensive groups. Endothelial dysfunction may lead to impaired insulin action and capillary leakage of albumin, which may be linked to a predisposition to cardiovascular disease. Thus, in addition to being an early marker of incipient diabetic nephropathy, urinary albumin excretion is closely linked to vascular endothelial function by mechanisms that may represent common pathways for the development of vascular disease (21).

PAI-1, the most important fibrinolytic system regulator, inhibits tissue plasminogen activator and prevents its interaction with plasminogen. Fibrinolytic activity at any site of vasculature is largely determined by the local balance of plasminogen activators and inhibitors (22). Hypertension, insulin resistance, type 2 diabetes, and postmenopause increased the risk of cardiovascular disease and elevated PAI-1 levels (23). In our study PAI-1 levels of the hypertensive, diabetic hypertensive, and diabetic normotensive groups were significantly higher than those of the control group. Circulating PAI-1 is also produced by other tissues like liver and adipose tissue. In our study, similar to PAI-1 levels, BMI of hypertensive, diabetic hypertensive, and type 2 diabetic patients was significantly higher than that of the control group. According to multiple regression analysis, our data showed no effect of BMI on PAI-1 levels.

In our study there were no significant differences between the groups, but in the hypertensive and diabetic hypertensive groups thrombomodulin levels were slightly lower. Salomaa et al. stated that increased thrombomodulin levels were thought to reflect endothelial damage, and among healthy individuals the risk of coronary heart disease gradually decreased with increased soluble thrombomodulin (24). High concentrations of thrombomodulin may indicate a low prothrombotic state and lower risk of coronary heart disease. Sadawa et al. showed that plasma thrombomodulin levels may decrease with hypertension in DOCA induced hypertensive rats (25). Because of overlapping results of thrombomodulin in healthy and endothelial damaged individuals, PAI-1 may be a better endothelial damage marker than thrombomodulin.

Increased levels of adhesion molecules have been found in human hypertension (26) and diabetes mellitus (27). In our study both VCAM-1 and PECAM-1 were higher in patients than in controls but not significantly. It has been demonstrated that adhesion molecules of the immunoglobulin superfamily (ICAM-1 and VCAM-1) are poorly expressed by the resting endothelium, but they are upregulated in patients at high risk of developing atherosclerosis, such as diabetic patients. Early endothelial activation and damage might be present in hypertensives, diabetic hypertensives, and diabetics.

The renin-angiotensin system (RAS) plays a pivotal role in the pathogenesis of insulin resistance and cardiovascular disease in diabetics. Interruption of RAS with ACE inhibitors and ARBs has been shown to prevent the onset of diabetes in hypertensive patients. Recently, many studies have been carried out to describe the endothelial effects of ARBs used for blood pressure regulation because hypertension is one of the most important causes of endothelial dysfunction. In the LIFE trial the incidence of new onset type 2 diabetes mellitus was reported to be significantly lower in hypertensive subjects treated with losartan than in those treated with atenolol (28). In the VALUE trial the incidence of new-onset type 2 diabetes mellitus was observed to be significantly lower in hypertensive subjects treated with valsartan than in those treated with amlodipine (29). In the CHARM Preserved trial the incidence of new-onset type 2 diabetes was significantly lower in subjects given candesartan than in those given placebo (30). However, in the other placebo-controlled trials including CHARM Alternative (31) and CHARM Added (32) there was no significant difference. In secondary prevention, the ONgoing Telmisartan Alone in combination with Ramipril Global Endpoint Trial (ONTARGET) Study (33) enrolled 25,620 patients over the age of 55 years with coronary heart disease or diabetes, plus additional risk factors, but without evidence of heart failure. Telmisartan alone was found to be equally effective in reducing the primary outcome of cardiovascular death, stroke, heart attack, or hospitalization for new-onset heart

failure, as well as each component of this composite endpoint (34).

There is clinical evidence that telmisartan has favorable metabolic effects. Telmisartan 80 mg has an insulin sensitizing effect (35). In patients with type 2 diabetes mellitus treated with low dose telmisartan (40 mg once daily) improved plasma lipid profiles were seen but telmisartan did not improve glycemic control (36). In our study low dose telmisartan treatment had no significant effect on lipid profile. The Diabetics Exposed to Telmisartan And enalaprIL (DETAIL) study compared the effects of the ARB telmisartan 80 mg and the ACE inhibitor enalapril 20 mg in 250 type 2 diabetic patients with early nephropathy. Telmisartan provided long-term renoprotection (37). In Kulkarni et al.'s pilot study telmisartan was effective, safe, and well tolerated, while reducing microalbuminuria in adult Indian hypertensive patients with type 2 diabetes mellitus (38). In our study, 40 mg telmisartan did not decrease urinary albumin excretion in 12 weeks and patients should be observed for a longer period. At the beginning of our study we postulated that a low dose of telmisartan may regulate blood pressure and have favorable effects on endothelial dysfunction. The low dose of telmisartan significantly lowered blood pressure but had no significant effect on FMD. All these data suggested that regulation of blood pressure by 40 mg/day telmisartan improved endothelial dysfunction minimally and the mechanisms were obscure. It is not clear whether it is the result of blood pressure regulation or the molecular efficiency of telmisartan.

Telmisartan, unlike other ARBs, acts as a partial PPARγ agonist at concentrations that are achievable with oral doses recommended for the treatment of hypertension (6). Hypertension and diabetes mellitus together frequently increase the risk of cardiovascular events. Telmisartan with a partial PPAR-γ effect may improve endothelial dysfunction. PPARγ agonist properties of telmisartan may help in the prevention of atherosclerosis (39).

The main limitation of our study was that a small number of patients were investigated. Further studies should be designed in larger groups to investigate the effects of telmisartan on endothelial dysfunction. Furthermore, to investigate the endothelial effects of telmisartan, different treatment protocols such as 40 mg/day or 80 mg/day may also be compared.

Conclusion

In this study we once again showed that type 2 diabetes mellitus and essential hypertension result in endothelial dysfunction. Moreover, 40 mg/day telmisartan significantly raised PON1 levels in both diabetic hypertensive and hypertensive patients and TM levels in diabetic hypertensive patients but there were no significant changes in other biochemical parameters or vascular analysis. All these data indicate that, although 40 mg/day telmisartan decreased blood pressure to normal ranges, it has a minimal role in improvement of endothelial dysfunction.

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