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The effect of nephropathy on plasma sphingosine 1-phosphate concentrations in patients with type 2 diabetes

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ABSTRACT

Objectives: Sphingosine 1-phosphate (S1P) is carried in plasma by the HDL particles and albumin. It mediates several protective functions of HDL. Because of its barrier-enhancing effect, it has attracted attention in diseases associated with endothelial dysfunction. We examined the impact of circulating levels of S1P in diabetic nephropathy together with apoprotein M, a S1P-binding protein in HDL. Plasma levels of dimethylarginines were evaluated in this context.

Design and methods: Patients with type 2 diabetes mellitus were divided into three groups according to daily albumin excretion: normoalbuminuria, microalbuminuria and macroalbuminuria ($n = 30$ in each). In addition to routine analysis, S1P and apo M in plasma were measured using the enzyme-linked immunosorbent assays. Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine were determined by HPLC. Tukey's or Mann–Whitney U-test was used for the statistics.

Results: Plasma S1P levels showed a significant decline in parallel to kidney dysfunction. The highest significance was detected in the macroalbuminuric group. Although a significant increase in plasma SDMA in albuminuric groups was observed, apo M, L-arginine and ADMA levels were similar between the groups.

Conclusion: Low plasma levels of S1P seemed to be associated with diabetic nephropathy. The main reason for the decreased S1P levels in our patients seems to be severe urinary albumin loss due to nephropathy. Low levels of S1P in patients with nephropathy may adversely affect the endothelial integrity and barrier function, thus causing a vicious circle.

1. Introduction

It is known that long-term exposure to high plasma glucose leads to a deterioration in glomerular capillary layers including endothelial glycocalyx, and results in proteinuria [1]. Considering the pivotal role of increased endothelial permeability in the progression of diabetic complications such as nephropathy, protection of endothelial barrier function is essential in the therapy of persons with diabetes.

Sphingosine 1-phosphate (S1P), a pleiotropic lipid mediator, is produced by the phosphorylation of sphingosine via sphingosine kinases (SKs) in response to a variety of stimuli. S1P binds to five related G-protein-coupled receptors, termed $S1P_1$–$S1P_5$. Many reports have demonstrated the critical role of S1P signaling in the maintenance of vascular barrier integrity [2–6]. In experimental animals deficient in S1P, increased vascular permeability and mortality were observed after induction of anaphylaxis by exposure to histamine, thrombin and lipopolysaccharide (LPS) [7]. In addition, an important role of S1P signaling in the regulation of lymphocyte egress from thymus and secondary lymphoid organs was also emphasized. The low S1P signaling is related to profound lymphopenia, thereby increasing sensitivity to infections [8].

Several candidates for the generation of S1P in blood have been identified, including erythrocytes, platelets and endothelial cells [2]. Among these, erythrocytes appear to be a major contributor to the supply of plasma S1P due to the lack of S1P-degrading enzymes [9].

In plasma, S1P is carried by HDL (~65%) or albumin (~35%) [10,11]. Albumin and HDL-associated S1P may be considered as being "bioactive" since it is able to activate S1P receptors. Albumin and HDL not only bind S1P, but also trigger the release of S1P from erythrocytes [9,12]. S1P is a key mediator of some protective effects of HDL such as vasorelaxation and barrier-promoting action [13–15] Apoprotein M (apo M), a lipocalin in the plasma HDL fraction, has recently been identified as the physiological carrier of HDL-associated S1P in vivo [15]. Being a carrier of S1P, apo M takes part in the vasoprotective action of HDL on endothelium.

The exact role of S1P in kidney physiology and pathophysiology remains unclear. It seems possible to suggest that because of the barrier-promoting action and vasorelaxation, S1P signaling may have a positive impact on kidney function. Indeed, administration of $S1P_1$ receptor agonists in experimental diabetic models has been reported

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to attenuate renal dysfunction and albuminuria [16], and improve coronary microvascular function [17]. It has been found that the level of S1P in HDL was decreased in patients with diabetes who had elevated levels of HbA1c [18]. In a clinical study, the expressions of $S1P_1$ receptor and S1P processing enzymes in vitreous humor have been found to be inversely associated with proliferative diabetic retinopathy [19].

Another factor that could affect the integrity of endothelium is bioavailability of nitric oxide (NO). NO is generated from L-arginine by the enzyme nitric oxide synthase (NOS). Asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, is considered as a causative factor for endothelial dysfunction. ADMA and its stereoisomer symmetric dimethylarginine (SDMA) are produced during post-translational methylation of arginine residues by protein arginine methyl transferases (PRMTs) in proteins and subsequently released by proteolysis [20]. Owing to its inhibitory effect on NO synthase, ADMA appears to be an important causative factor in cardiovascular diseases [21], whereas SDMA influences NO bioavailability only by inhibiting arginine uptake [22]. The metabolic fate of these isomers is different, too. SDMA is fully eliminated via kidney and is strongly related to renal function [23], while ADMA is mainly degraded in liver and kidney by dimethylarginine dimethylaminohydrolase (DDAH).

In patients with non-diabetic kidney disease, circulating ADMA levels have been demonstrated to be positively correlated with the degree of albuminuria [24]. In patients with diabetic proteinuria, accumulation of ADMA in the circulation has been reported [25]. Whether the increase in ADMA levels is due to impaired renal function or among the causative factors of renal impairment has not been elucidated yet.

In this work, we wanted to examine the impact of circulating levels of S1P and apo M in diabetic nephropathy. Plasma levels of L-arginine, ADMA and SDMA were evaluated in this context.

2. Materials and methods

The persons with diabetes who attended their annual screening visit for diabetic complications to the Division of Endocrinology and Metabolism, Department of Internal Medicine, were invited to participate in this study. Ninety patients with type 2 diabetes mellitus were divided into three groups according to their urinary albumin excretion rate as follows: normoalbuminuria (<30 mg/day), microalbuminuria (30–300 mg/day) and macroalbuminuria (>300 mg/day). Macrovascular disease was defined as evidence of ischaemic heart disease, stroke or peripheral vascular disease. Diagnosis of retinopathy was based on ophthalmoscopy. The diagnosis of diabetic neuropathy is based on symptoms, medical history and physical examination. This study was approved by the Ethics Committee for Human Studies, Istanbul Faculty of Medicine, and all patients signed an informed consent. The study conformed to the Declaration of Helsinki.

Serum glucose, HbA1c, blood urea nitrogen (BUN), creatinine levels and lipid profile were determined by an automated analyzer [Cobas Integra 8000 (c702) chemistry analyzer: Roche Diagnostics]. Hematological parameters in plasma with EDTA were measured by an automated analyzer (Beckman Coulter LH 780 Hematology Analyzer).

To analyze high-sensitivity C-reactive protein (hs-CRP) and urinary albumin, we used high sensitive immunoturbidimetric assays (Cobas Integra 6000 (c501) chemistry analyzer: Roche Diagnostics).

The glomerular filtration rate (GFR) was calculated as GFR [mL/min (1.73 m^2)] = 186 / $creatinine^{1.154}age^{0.203}$ in males, and GFR = 138 / $creatinine^{1.154}age^{0.203}$ in females [26].

The concentrations of asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine in plasma samples with EDTA were determined with high performance liquid chromatography (HPLC) fluorometric method after samples had been treated with o-phtaldialdehyde to convert methylarginines to a fluorescent compound [27].

The concentrations of sphingosine 1-phosphate (MyBiosource Inc. San Diego, CA, USA) and apoprotein M (Cloud-Clon Corp. Houston, TX, USA) were measured in plasma with EDTA using an enzyme-linked immunosorbent assay. The lower detection limit of S1P was 2.34 ng/L. Both intra-assay CV (%) and inter-assay CV (%) is less than 15%.

Serum albumin was measured by a dye-binding technique using bromocresol green (BCG) [28].

For the statistical analyses, One-way of variance (ANOVA) followed by Tukey's honestly significant difference was used for equal variances. The Kruskal–Wallis test (post-hoc Mann–Whitney U) was performed for unequal variances. Pearson's and Spearman's correlation coefficients (r) were computed to explore the correlation between two variables. If variables were not normally distributed, it was log-transformed prior to correlations and it was checked whether the transformed data have suitable distribution for the statistical methods chosen.

3. Results

The clinical characteristics of the patients with or without nephropathy are shown in Table 1. Duration of diabetes was longer in patients with albuminuria as compared with counterparts with normoalbuminuria. Patients with macroalbuminuria had more complications. The frequency of cardiovascular disease (50%), retinopathy (70%) and neuropathy (67%) was highest in this group. A greater proportion of these patients was receiving insulin therapy (67%) and antihypertensive drug (97%). Despite intense antihypertensive therapy, blood pressure in patients with macroalbuminuria was higher than in the other two groups. Glycemic status of persons with diabetes was comparable in three groups (Table 2). Although a mildly hypertriglyceridemia (>1.69 mmol/L, upper limit of reference value) was observed in all persons with diabetes, LDL cholesterol (<3.37 mmol/L) and HDL cholesterol (>1.03 mmol/L) levels were within normal limits. No difference in plasma lipid parameters was found between patient groups. As compared with patients with normoalbuminuria, plasma levels of creatinine, BUN and SDMA were higher in patients with albuminuria, being highest in group with macroalbuminuria (Table 2). Similarly, the reduction in GFR was most pronounced in group with macroalbuminuria.

Hematocrit, hemoglobin and platelet counts as well as plasma albumin levels were also found to be lower in patients with macroalbuminuria. Elevated CRP levels in this group indicated an ongoing inflammation.

Although a significant increase in plasma SDMA levels in the group with macroalbuminuria was observed, L-arginine and ADMA remained unaltered. We found the plasma ADMA and SDMA levels of healthy control as 0.69 (80.5–0.95) μmol/L and 0.43 (0.19–0.84) μmol/L, respectively. These were in accordance with the reference values in the previous study [29].

Table 1
Some characteristics of the diabetic patients.

	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
Number of subjects	30	30	30
Male/female	12/18	17/13	16/14
Age (years)	54.2 ± 1.32	59.4 ± 1.53[a]	61.8 ± 1.36[a]
Duration of diabetes (years)	9.3 ± 1.2	12.7 ± 1.5[a]	14.1 ± 1.3[a]
Cardiovascular disease (%)	6 (20)	8 (27)	15 (50)[a]
Retinopathy (%)	2 (7)	12 (40)[a]	21 (70)[a,b]
Neuropathy (%)	11 (37)	12 (41)	20 (67)[a,b]
Insulin (%)	11 (37)	10 (37)	20 (67)[a,b]
Antihypertensive drug (%)	23 (77)	26 (87)	29 (97)[a]
Statins (%)	17 (57)	20 (67)	23 (77)
Systolic BP (mm Hg)	120.3 ± 1.89	118.4 ± 1.59	128.1 ± 1.49[a,b]
Diastolic BP (mm Hg)	75.86 ± 1.36	74.82 ± 1.27	78.50 ± 1.10[b]

Categorical variables are expressed as number in each category and percentages given in parentheses; continuous variables are expressed as mean ± SE.
[a] $p < 0.05$ as compared with normoalbuminuric patients.
[b] $p < 0.05$ as compared with microalbuminuric patients.

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