Plasma Mature Form of Adrenomedullin in Diabetic Nephropathy

Key words: hypertension, renal insufficiency diabetes mellitus, PAMP

Adrenomedullin (AM) is a potent vasodilative and natriuretic peptide that is processed from its precursor as the intermediate form, AM-glycine-COOH (iAM) (1). Subsequently, iAM is converted to the biologically active mature form, AM (1-52)-CONH₂ (mAM) by enzymatic amidation (1). It is reported that AM also acts as an antiproliferative factor on endothelial and mesangial cells (2). One of the major complications of diabetes mellitus is nephropathy, which is strongly connected with vascular damage and mesangial alterations. Further studies to clarify the mechanism in the development and progression of nephropathy are needed to prevent end-stage renal failure. AM seems to be a candidate that plays some role through its hypotensive and antiproliferative effect. It is also reported that hyperglycemia enhances AM expression in the vessels, indicating that AM is involved in the regulation of glycemic control (3). The plasma total AM (tAM: mAM and iAM) level is correlated with mean blood pressure (MBP) in diabetic patients without nephropathy (4). However, the behavior of AM in diabetic nephropathy and the mAM levels in diabetes are as yet unknown.

Thirty-seven patients with type 2 diabetes mellitus (mean age; 62.2±2.2 years [SEM], 22 men and 15 women) were classified into four groups: 14 patients with normoalbuminuria served as controls (Normoalbuminuria group), 8 patients with

overt proteinuria and serum creatinine less than 1.2 mg/dl (Proteinuria group), 7 patients with chronic renal failure whose creatinine levels were more than 1.3 mg/dl (CRF group) and 8 patients undergoing hemodialysis (HD group). Blood samples were drawn from the antecubital vein into chilled polypropylene tubes containing EDTA-2Na (1 mg/ml of blood) and aprotinin (500 units/ml of blood) between 8:30 AM and 10:00 AM after an overnight fast. From HD patients blood was taken just before the start of HD. Plasma concentrations of mAM and tAM were measured using the following kits: AM mature RIA SHIONOGI and AM RIA SHIONOGI (Shionogi Co., Osaka).

Table 1 shows the clinical characteristics of the study groups. There were no significant differences in age, plasma glucose, HbA1c, or body mass index (BMI) between the groups. The levels of serum creatinine and plasma tAM in CRF group were significantly higher than those in the Normoalbuminuria group. Serum creatinine levels and plasma concentrations of tAM and mAM in HD group were significantly higher than those in the other three groups. MBP was statistically higher in HD group than in Normoalbuminuria and Proteinuria groups. The plasma concentrations of tAM and mAM in all participants were 17.3±1.4 and 2.0±0.3 fmol/ml. The plasma tAM and mAM concentrations were significantly correlated with the values for serum creatinine ($r=0.85$, $p<0.001$ and $r=0.82$, $p<0.001$) and MBP ($r=0.38$, $p<0.05$ and $r=0.41$, $p<0.01$), but not with those for age, plasma glucose, and HbA1c and BMI.

The early renal abnormalities in diabetic nephropathy include glomerular hypertension and hyperfiltration. Sustained glomerular hypertension together with several other factors, such as advanced glycation end-products, cytokines, hyperlipidemia, sorbitol, angiotensin II and growth factors, causes thick-

Table 1. Clinical Characteristics of the Study Groups

	Normo- albuminuria	Proteinuria	CRF	HD
Number	14	8	7	8
Age (years)	59.2±3.4	64.5±4.8	60.1±4.0	66.1±6.0
Sex (M/F)	7/7	5/3	5/2	5/3
Plasma glucose (mg/dl)	130.1±8.4	161.1±25.0	175.1±33.2	165.4±2.7
HbA1c (%)	6.4±0.3	6.8±0.6	6.7±0.9	6.4±0.3
Serum creatinine (mg/dl)	0.74±0.04	0.84±0.06	4.1±1.1 ^a	7.8±0.6 ^b
MBP (mmHg)	93.1±2.5	92.4±4.6	107.3±3.8	115.1±5.1 ^c
BMI (kg/m ²)	22.1±0.9	23.3±1.9	21.4±0.8	20.5±0.6
Plasma tAM (fmol/ml)	11.2±0.6	13.9±1.0	18.1±1.4 ^d	30.7±2.9 ^b
Plasma mAM (fmol/ml)	1.1±0.1	1.0±0.1	2.0±0.4	4.6±0.7 ^b

Values are expressed as means±SE. MBP indicates mean blood pressure; BMI: body mass index, tAM: total adrenomedullin, mAM: mature adrenomedullin. ^a $p<0.001$ compared with normoalbuminuria, $p<0.01$ compared with proteinuria, ^b $p<0.001$ compared with normoalbuminuria, proteinuria and CRF, ^c $p<0.01$ compared with normoalbuminuria and proteinuria, ^d $p<0.05$ compared with normoalbuminuria.

ening of glomerular basement membrane and mesangial expansion eventually followed by glomerulosclerosis. AM induces vasodilation in renal arteries, increases the glomerular filtration rate, and regulates renin secretion. It is suggested that AM inhibits the proliferation of endothelial and mesangial cells (2). These previous studies suggest that AM is a candidate as a regulator in the progression of nephropathy. In the present study, the plasma AM level was significantly correlated with serum creatinine and MBP. The increased plasma AM level in renal insufficiency may be partly due to decreased clearance in the kidney, in spite of the fact that circulating AM is greatly metabolized in the pulmonary circulation (5). The vascular endothelial damage and volume retention in nephropathy could be other reasons for the elevated plasma AM levels (6). Taken together with the vasodilative effect in renal arteries, the production of AM may be augmented to preserve renal blood flow and glomerular filtration rate when renal function deteriorates. Plasma mAM level in CRF was higher than that in Proteinuria group, but this is not statistically significant. More studies are needed to confirm the role and behavior of mAM in renal insufficiency. The significant correlation between plasma AM level and MBP suggests that AM participates in the regulation of blood pressure, which is consistent with our previous data found in diabetic patients without nephropathy (4).

This is the first study that analyzes plasma AM levels in diabetic nephropathy using two newly developed immunoradiometric assays. We have shown that plasma tAM and mAM levels are elevated as the severity of diabetic nephropathy increases and these levels also correlate with MBP. Considering

its potent renal vasodilative effects and antiproliferative effects, AM may play some role in diabetic nephropathy.

Hiroshi KINOSHITA, Shouichi FUJIMOTO, Takeshi TOKURA,
Shuichi HISANAGA, Kazuo KITAMURA and Tanenao ETO

From the First Department of Internal Medicine, Miyazaki Medical College, Miyazaki

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Reprint requests should be addressed to Dr. Hiroshi Kinoshita, the First Department of Internal Medicine, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-1692

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