Plasma Concentrations of Adrenomedullin and Ghrelin in Hemodialysis Patients with Sustained and Episodic Hypotension

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Abstract. Sustained and/or episodic hypotension during hemodialysis (HD) is an important clinical issue. Plasma adrenomedullin (AM) is increased in HD patients with sustained hypotension, but little is known about its implications for episodic hypotension. Ghrelin may also contribute to the pathophysiology of hypotension in HD patients. We evaluated plasma levels of AM and total ghrelin in sustained hypotensive (SH; n = 23), episodic hypotensive (EH; n = 30) and normotensive (NT; n = 23) HD patients. In the EH group, the relationship between low blood pressure during HD and circulating levels of AM and ghrelin was also evaluated. Plasma levels of AM were significantly higher in SH (34.3 ± 8.3 fmol/ml, p<0.01) than in NT patients (27.6 ± 5.2 fmol/ml), but not in EH patients (30.8 ± 6.1 fmol/ml). There was no significant difference of plasma total ghrelin in SH (548.1 ± 426.5 fmol/ml) and in EH patients (544.6 ± 174.3 fmol/ml), compared with NT patients (400.0 ± 219.7 fmol/ml). On the other hand, in EH patients, the “suppressed blood pressure ratio” during HD significantly correlated with plasma AM (r = 0.77, p<0.001) and with total ghrelin levels (r = 0.44, p<0.05). Our results suggest that ghrelin, as well as AM, may play an important role as vasodilator local hormones and regulation of blood pressure during HD, especially the occurrence of EH. Further studies are necessary to clarify the implication of these hormones in the control of hypotension during HD.

Key words: Adrenomedullin, Chronic hypotension, Episodic hypotension, Ghrelin, Hemodialysis, Sustained hypotension

SIGNIFICANT hypotension is a major cardiovascular complication in patients with end-stage renal disease undergoing hemodialysis (HD). Two types of hypotension are recognizable in the setting of maintenance HD: episodic hypotension (EH) during HD is the most common manifestation of hemodynamic instability, and occurs in around 30–40% of the dialysis population [1]. A second form is sustained hypotension (SH), characterized by a systolic blood pressure (SBP) lower than 100 mmHg, during the interdialysis period and is present in approximately 5–10% of patients [2, 3]. Both groups of patients require a substantial amount of medical and nursing care during and after HD to control hypotension-related symptoms. Although several clinical factors, such as autonomic dysfunction, reduced pressor response to vasopressor agents and cardiac dysfunction, have been shown to be responsible for the occurrence of EH and SH [1], the pathophysiology of chronic hypotension in dialysis patients has yet to be fully clarified.
Adrenomedullin (AM) is a novel vasodialator peptide that has been recently isolated from human pheochromocytoma cells by monitoring the elevated activity of platelet cyclic adenosine monophosphatase [4]. AM is present in several normal tissues, such as adrenal medulla, lungs, kidneys and cardiac atrium, and has a potent and long-lasting hypotensive effect [5]. In 2000, Cases et al. [6] reported that plasma AM levels and nitrate levels were increased in HD patients, but only AM levels were higher in sustained hypotensive than in normotensive (NT) and hypertensive HD patients. Based on these results, they suggested the involvement of AM in the pathophysiology of SH in HD patients. Although several studies supported the association between AM and SH, there is no report to describe the association between AM and EH, which is more frequent among the dialysis population.

Ghrelin is a novel growth hormone (GH)-releasing peptide, originally isolated from the rat stomach, which was identified as an endogenous ligand for an orphan receptor termed GH secretagogue receptor [7]. Human ghrelin is a 28-amino acid peptide with a fatty acid chain modification on the N-terminal third amino acid. The hydroxy atom of the hydroxyl group of the N-terminal third amino acid serine residue is replaced by a hydrophobic moiety, C7H15CO; in other words, the hydroxyl group of Ser3 is octanoylated [7]. The n-octanoyl group at this position of the ghrelin molecule seems to be essential for some of the hormone’s activity, including GH release and appetite. Non-acylated (desoctanoyl or desacyl) ghrelin circulates in far greater amounts than the acylated form and does not displace ghrelin from its hypothalamic and pituitary binding site [7].

Ghrelin is synthesized in several organs, such as intestine and kidney, as well as stomach [8, 9]. Recent studies reported that peripheral administration of ghrelin or GH secretagogue causes not only GH release from the pituitary gland, but also improvement in cardiac function, increase in food intake, fat accumulation and decrease in blood pressure [10, 11]. Furthermore, Wiley and Davenport investigated the vasodilator function of ghrelin by using human internal mammary artery in vitro, and concluded that ghrelin was an effective, endothelin-independent vasodilator of the long-lasting constrictor endothelin-1 in human arteries producing responses similar to those of AM [12]. From this point of view, it is speculated that accumulated ghrelin, as well as AM, before dialysis may cause hypotensive status in HD patients through its vasodilator effect. However, to date there is no study to clarify the association between plasma ghrelin concentration and SH and/or EH.

Based on the above background, we hypothesized that increased production of AM and ghrelin might be the underlying mechanism of SH and EH in HD patients. This study was designed to assess the possible role of AM and ghrelin in the pathogenesis of SH and EH in hemodialyzed patients.

Methods

Study participants

The study subjects were 76 patients with renal diseases on maintenance HD (23 SH, 30 EH and 23 NT patients). SH was defined as SBP less than 100 mmHg at predialysis in at least 80% of blood pressure measurements in the previous three months [1]. EH was defined as decreases of more than 25 mmHg during HD and/or as hypotension requiring medication during HD [13]. NT was defined as SBP less than 145 mmHg and DBP less than 90 mmHg at predialysis at least 80% of blood pressure measurements in the previous three months [1]. The causes of renal disease were chronic glomerulonephritis (n = 61), diabetic nephropathy (n = 12), Crohn’s disease (n = 1) and undefined (n = 2). None of the patients were anephric, had evidence of cardiac disease, such as myocardial infarction, or suffered from chronic obstructive pulmonary disease or hepatic dysfunction. None of the patients received antihypertensive treatment and vasodilatory drugs. Before the study, ethical approval was obtained from the special committee of Nagasaki University School of Medicine (project registration no. 15052224). Blood samples from patients were collected at three hospitals in Nagasaki city (see Acknowledgment). In all cases, a signed informed consent was obtained before the study.

Measurement of plasma AM and ghrelin concentrations

Blood samples were collected in tubes with 2 mg/ml of ethylenediaminetetraacetic acid (EDTA)-2Na and 500 KIU/ml aprotinin just before the dialysis. After collection, the samples were promptly centrifuged at 4°C. Plasma total AM was measured by immunoradiometric assay using a specific kit for each form
Plasma total ghrelin was measured by our specific radioimmunoassay (RIA) system as described previously [9]. Since the active form of ghrelin is unstable in non-acidified normal plasma, the total amount of ghrelin was used in this study.

We defined “suppressed blood pressure ratio” in patients with EH as [(systolic blood pressure at predialysis) – (minimum systolic blood pressure at the episode of hypotension during HD)/systolic blood pressure at predialysis] × 100 (%), and determined its correlation with plasma levels of AM and ghrelin.

**Statistical analysis**

Statistical analysis was performed using software package SPSS 9.0 for Windows (Chicago, IL). Data are expressed as means ± SD. One-way ANOVA was used for statistical comparisons between SH and NT groups, and EH and NT groups. Pearson correlation analysis was performed for correlation between variables, and a p value less than 0.05 was accepted as statistically significant.

**Results**

Table 1 summarizes the hemodynamic and laboratory values for the three groups of HD patients. Systolic, diastolic, and mean blood pressures were significantly different between SH and NT patients, and EH and NT patients (p<0.001 and p<0.001, respectively). On the other hand, there was no significant difference between SH and EH patients. Duration of HD was significantly longer in SH than in NT patients (p<0.001). There was no significant difference of hematocrit (Ht) in SH (34.5 ± 8.1%) and in EH patients (31.8 ± 3.2%), compared with NT patients (30.9 ± 2.4%).

When data of all patients were analyzed, plasma levels of AM correlated with those of ghrelin (r = 0.29, p = 0.003, Fig. 1). Plasma levels of AM in HD patients were significantly higher in SH (34.3 ± 8.3 fmol/ml, p<0.01) than in NT patients (27.6 ± 5.2 fmol/ml, Table 1), but not in EH patients (30.8 ± 6.1 fmol/ml). There was no significant difference of plasma total ghrelin in SH (548.1 ± 426.5 fmol/ml) and in EH patients

| Table 1. Demographic, hemodynamic, and laboratory values of the three groups of HD patients. |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Number of patients                           | 23              | 30              | 23              |
| Males (%)                                     | 30.4            | 53.1            | 69.5            |
| Age (years)                                   | 57.3 ± 12       | 56.7 ± 14.7     | 64.4 ± 13.8     |
| Duration of HD (years)                        | 18.7 ± 11.1b    | 10.6 ± 8.3      | 8.0 ± 5.4       |
| Systolic BP (mmHg)                            | 87.1 ± 11.6b    | 104.1 ± 11.5b   | 119.1 ± 16.6    |
| Mean BP (mmHg)                                | 62.1 ± 8.5b     | 79.4 ± 12.2b    | 94.1 ± 8.7      |
| Diastolic BP (mmHg)                           | 49.5 ± 7.9b     | 64.0 ± 13.7b    | 71.5 ± 8.9      |
| Interdialysis weight gain (g)                 | 1218.3 ± 1102.7 | 2690.7 ± 1221.5 | 2269.7 ± 993.4 |
| Hematocrit (%)                                | 34.5 ± 8.1      | 31.8 ± 3.2      | 30.9 ± 2.4      |
| Plasma AM (fmol/ml)                           | 34.3 ± 8.3a     | 30.8 ± 6.1      | 27.6 ± 5.2      |
| Plasma total ghrelin (fmol/ml)                | 548.1 ± 426.5   | 544.6 ± 174.3   | 400.0 ± 219.7   |

Data are mean ± SD.

*p<0.01, 'p<0.001, vs normotensive patients.
HD, hemodialysis; BP, blood pressure.
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(544.6 ± 174.3 fmol/ml), compared with NT patients (400.0 ± 219.7 fmol/ml). On the other hand, in EH patients, the “suppressed blood pressure ratio” significantly correlated with plasma levels of AM (r = 0.77, p<0.001) and of ghrelin (r = 0.44, p<0.05, Fig. 2). On the other hand, there was no correlation between “suppressed blood pressure ratio” and Ht.

Discussion

Our study showed that plasma AM levels are significantly increased in patients with SH. In addition, we showed that in patients with EH, plasma AM levels correlated positively with the “suppressed blood pressure ratio”. Furthermore, we showed for the first time that the levels of plasma total ghrelin also correlated with the “suppressed blood pressure ratio” in EH patients. These results suggest that as vasodilator peptides, these two hormones may be involved in the pathophysiology of hypotension in HD patients, especially in EH.

Several groups have investigated the mechanism(s) of high levels of vasodilator agents during HD [1, 14–16]. Imai et al. [14] conducted a hemodynamic study and reported that while cardiac index, heart rate or stroke volume were similar in hypertensive and normotensive HD patients, total peripheral vascular resistances were lower in the former group. They suggested that increased biosynthesis and/or release of vasodilator agents might be critical in the pathogenesis of hypotension during HD. Plasma atrial natriuretic peptide levels have been reported to be similar in hypertensive and normotensive dialysis patients [15, 16], but the possible role of this molecule in chronic hypotension in uremia is controversial [6]. In addition, it has been shown that plasma levels of adenosine, a strong hypotensive agent, are increased, while the activity of intracellular adenosine deaminase, the enzyme that metabolizes this agent, is reduced in HD patients, but not in predialysis or peritoneal dialysis patients [17]. However, the possible role of this agent in hypotension in dialysis patients has yet to be fully evaluated.

In addition to these peptides, AM was reported to be increased in patients with SH [6, 18]. However, there is little or no information regarding the relationship between AM and EH. In the present study, we found that plasma AM levels are increased in patients with SH. In addition, we showed that AM levels positively correlated with the “suppressed blood pressure ratio” in patients with EH. Thus, the present results suggest that AM may be involved in the pathophysiology of EH and SH in HD patients.

The exact mechanism of the increased production of vasodilators including AM is still unknown. However, it is likely that the inflammatory state of uremia plays some role [19]. The production of both nitric oxide and AM is induced by cytokines, such as hepatocyte growth factor (HGF), which induces endothelial proliferation and nitric oxide-mediated vasodilation, and other studies showed that HGF was increased in hypertensive HD patients [20]. Several studies also suggested the possible roles of microinflammatory state in chronic hypotension of dialysis patients, through the
induction of synthesis of several vasodilator substances [21, 22]. Although further studies are needed, similar mechanism(s) may be associated with the pathophysiology of EH during dialysis.

Besides vasodilator effect, it is suggested that AM may also be associated with circulating blood volume in HD patients [23]. In our current study, there was no relationship between Ht, one of the markers of circulating blood volume, and hypotension. Also there was no relationship between Ht and AM. Further studies will be needed to clarify the contribution of AM to EH and SH through the change of blood volume in HD patients.

Ghrelin, an endogenous peptide recently linked to growth hormone secretagogue receptor [7], is a potent, endothelium-independent vasodilator of human arteries, effectively reversing endothelin-1 (ET-1)-mediated constriction. Ghrelin is present in human plasma at approximately 100 pmol/L [7], a concentration considerably higher than other vasoactive peptides. Yoshimoto et al. demonstrated that plasma ghrelin in patients with renal disease is increased in parallel with the severity of renal damage [24]. They also revealed that approximately half of the plasma ghrelin, as well as half of the serum creatinine or blood urea nitrogen, are removed from the blood by a single course HD, and that bilateral nephrectomy in mice causes marked increase in plasma ghrelin concentrations. They concluded that increased plasma ghrelin in renal failure may result from decreased clearance or degradation in the kidney. Although overproduction of ghrelin in organs other than the stomach may contribute to higher plasma concentrations [25, 26], a similar pathophysiological changes may occur in patients in HD. In our current study, we showed a positive correlation between plasma AM and ghrelin concentrations in HD patients, and found a positive correlation between “suppressed blood pressure ratio” and plasma levels of ghrelin in EH patients. These results suggest that ghrelin, in cooperation with AM, may contribute to the development of EH through its vasodilatory effect. On the other hand, we could not reveal the significant difference of total ghrelin levels between SH and NT patients, and EH and NT patients. Although this may merely reflect our small sample size, ghrelin may not play an important role in the occurrence of hypotension in chronic state.

In addition to this effect, ghrelin is associated with changes in body composition in patients on HD. Ayala et al. investigated patients with end-stage renal disease and found markedly high plasma ghrelin concentrations in this group, and its level correlated significantly with plasma insulin, body mass index, log serum leptin levels and truncal fat mass [27]. These findings suggest that the kidney is an important site for clearance and/or degradation of ghrelin.

There are several limitations in this study. First, we measured AM and ghrelin only just before, not during and after HD. Observation on the dynamics of AM and ghrelin in a series of HD will be available to clarify the contribution of these hormones, more precisely. Second, we could not measure plasma levels of atrial natriuretic peptide and adenosine, which have been suggested to have possible roles in SH patients [19, 28]. Third, our sample size was relatively small to completely identify the roles of AM and ghrelin in SH and EH patients. Additional sampling may overcome the insufficient statistical significance in our study.

In this study, we observed that duration of HD was significantly longer in SH than in NT patients. It is known that longer duration of HD is associated with autonomic neuropathy, which is one of the major causes of hypotension in HD patients [29]. Besides vasodilator agents, such clinical factors should be also considered to be key factors for the occurrence of hypotension in HD patients. In conclusion, our results suggested the possibility that ghrelin and AM might play important roles as vasodilator local hormones and control of blood pressure during HD. Further studies are clinically important to clarify the implication of these hormones in the clinical control of hypotension during HD.

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References