Original Article

Association of Human Hepatocyte Growth Factor with Hemodialysis Hypotension

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Hypotension is a major cardiovascular complication of hemodialysis, and enhanced production of nitric oxide (NO) may be involved in hemodialysis hypotension. Human hepatocyte growth factor (hHGF), which induces endothelial proliferation, causes NO-mediated hypotension in animals. Because heparin, which is routinely used during hemodialysis, increases circulating hHGF concentration in humans, circulating hHGF may be involved in hemodialysis hypotension via increased NO production. To investigate the involvement of hHGF in NO production and hypotension in hemodialysis patients, we measured concentrations of serum hHGF and plasma NO3−, an index of endogenous NO production, in 114 patients undergoing maintenance hemodialysis. The mean serum hHGF concentration before dialysis was greater (p<0.01) in subjects with lower blood pressure (BP) (mean BP before dialysis ≤75 mmHg, n=16, 0.251±0.050 ng/ml) than in those with middle BP (mean BP before dialysis 76 to 109 mmHg, n=75, 0.143±0.016 ng/ml) or higher BP (mean BP before dialysis ≥110 mmHg, n=23, 0.088±0.017 ng/ml). The mean serum hHGF concentration after dialysis was higher in subjects with lower BP (1.854±0.242 ng/ml) than in those with middle BP (1.280±0.120 ng/ml) or higher BP (0.688±0.130 ng/ml). Serum hHGF concentration was positively correlated with plasma NO3− concentration (r=0.608, p=0.0001, n=114). Circulating hHGF may participate in the mechanism of chronic hemodialysis hypotension by affecting endogenous NO production.

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Key Words: hemodialysis, heparin, hepatocyte growth factor, hypotension, nitric oxide

Introduction

Hypotension is a major cardiovascular complication of hemodialysis (1). Two types of hypotension are recognized in the setting of maintenance hemodialysis: an episodic type, which occurs only during dialysis, and a chronic type in which hypotension persists in interdialysis periods as well as during dialysis (2). In an earlier study, we reported that inhibited sympathetic nervous activity is one of the causes of acute hypotension during dialysis, and that enhanced production of nitric oxide (NO) induced by cytokines such as interleukin-1 (IL-1) or tumor necrosis factor α (TNFα) is involved in this inhibition of sympathetic activity in patients having hypotensive episodes during dialysis (3). In the chronic type of hypotension, its mechanism is not precisely known; however, enhanced production of NO is also likely to be involved in chronic hemodialysis hypotension (4), although it is unknown whether either cytokine-induced NO or constitu-
tively released NO or NO from both sources plays a major role.

Human hepatocyte growth factor (hHGF), which is identical to scatter factor (5), is a disulfide-linked heterodimeric molecule composed of a 69-kD kringle-containing α-chain and a 34-kD β-chain (6). hHGF has multiple biologic activities as a mitogen and morphogen, and it may be involved in regeneration of the liver and kidney after injury, in atherosclerosis and arteriosclerosis (7), and in angiogenesis (8). Furthermore, hHGF inhibits cytokine-induced NO production (9), while IL-1, which is a major cytokine inducer of NO production in hemodialysis (10), increases hHGF synthesis (11). In addition, intravenous administration of hHGF elicits hypotension in rats, and increased release of NO is involved in this vasodepressor activity of hHGF (12). These findings indicate a possible involvement of hHGF in hemodialysis hypotension via regulation of NO production. In the present study, we measured the serum concentration of hHGF and plasma concentration of nitrate anion (NO$_3^-$), a stable index of endogenous NO production, to investigate the involvement of hHGF in the regulation of NO production and hypotension in subjects undergoing maintenance hemodialysis.

**Methods**

The study protocol was approved by the Ethical Committee for Human Research of Kyoto Prefectural University of Medicine. All subjects provided informed consent for participation in this study. The participants were 114 patients with end-stage renal disease who were undergoing 4-h (9:00-13:00) maintenance hemodialysis treatments three times a week at the hemodialysis center of Nishijin Hospital. The causes of renal failure included chronic glomerulonephritis (n = 70), diabetic nephropathy (n = 40), hypertensive nephrosclerosis (n = 2), and immunoglobulin A nephropathy (n = 2). None of the subjects had hepatic or renal diseases that would affect serum hHGF concentration. Any medications that could affect blood pressure (BP), such as anti-hypertensive drugs, synthetic erythropoietin, and α1-adrenoceptor agonists, were stopped 48 h before the start of this study; the suspension of medication with antihypertensive drugs did not significantly affect mean BP before dialysis in hypertensive patients (116 ± 4 mmHg vs. 118 ± 5 mmHg, p = 0.17). In addition, all participants were given nutritional counseling with a recommended daily protein intake of 50 g. To avoid the effects of diet and foods on BP, none of the participants had lunch during dialysis. BP was measured with the patients in a supine position with a standard sphygmomanometer by nurses every hour throughout dialysis. BP measurements were repeated at least three times, and the mean of the last two measurements was recorded as the actual BP. Upon beginning dialysis, all patients received a 1,000-U bolus dose of heparin followed by a continuous infusion of heparin during dialysis (500 U/h) for a total administration during hemodialysis of 3,000 U/patient. Hemodialysis was performed with a cuprophan dialyzer with an area of 1.5 m$^2$ (CL-S 15 N, Terumo, Tokyo), using a blood flow rate of 200 ml/min and a dialysate flow rate of 500 ml/min. The dialysate composition was Na$^+$ 140 mEq/l, K$^+$ 2.0 mEq/l, Ca$^{2+}$ 2.5 mEq/l, Mg$^{2+}$ 1.0 mEq/l, Cl$^-$ 114.5 mEq/l, CH$_3$COO$^-$ 8 mEq/l, HCO$_3^-$ 25 mEq/l, and glucose 150 mg/dl (Kindaly Solution AF-3P, Fuso Pharmaceutical, Osaka).

Participants were divided into the following three groups on the basis of their mean BP before dialysis measured for four consecutive weeks before this study: a lower BP group, in which the mean BP before dialysis was always less than 75 mmHg for four consecutive weeks (n = 16), a middle BP group, in which the mean BP before dialysis always ranged between 76 and 109 mmHg for four consecutive weeks (n = 75), and a higher BP group, in which the mean BP before dialysis was always more than 110 mmHg for four consecutive weeks (n = 23). The basic characteristics of these patient groups are shown in Table 1. The percentages of patients receiving medication of erythropoietin, which may affect BP in patients with end-stage renal diseases, were 75% in the lower BP group, 78% in the middle BP group, and 74% in the higher BP group. Blood (5 ml) was collected before and after dialysis; blood collected before dialysis was divided into tubes with or without EDTA-2Na to obtain serum and plasma samples, respectively, and that collected after dialysis was used to provide serum samples only. The serum and plasma samples were frozen at −30°C until hHGF, NO$_3^-$, urea nitrogen, and creatinine concentrations were measured. Serum concentrations of urea nitrogen and creatinine were measured with an automatic analyzer (Ektachem 700 analyzer, Eastman Kodak, Rochester, NY). Plasma NO$_3^-$ concentrations were measured only before dialysis, because circulating NO$_3^-$ is removed immediately by hemodialysis (3).

**Measurement of NO$_3^-$ Concentration**

The concentration of NO$_3^-$ (μmol/l) was determined by high-performance liquid chromatography (HPLC; LC-6A and 7A, Shimadzu Co., Kyoto) with an anion-exchange column (IC-Pak A, Waters, Milford, MA). Details of this method have been described elsewhere (3, 13, 14). In brief, the sample was deproteinized by passing it through a hydroxyapatite column (Pentax, Asahi Optical Co., Tokyo) and trapped in a concanavalin A column (IC-Conc A, Tosoh Co., Osaka). The trapped samples were released by elution with phosphate buffer (0.02 mM, pH 8.0, 1.2 ml/min) and introduced onto an analysis IC-Pak A column. Intra- and inter-assay coefficients of variance were 1.8 and 3.1%, respectively.
Measurement of hHGF Concentration

Serum hHGF concentrations were measured with a specific enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Tokyo); the intra- and interassay variations were 2.9% and 2.6%, respectively.

Statistical Analysis

Data are expressed as the mean ± SEM. Differences between values before and after dialysis in the same group were calculated with Student’s paired t test. Differences between groups of data were evaluated by performing an ANOVA followed by Duncan’s multiple range test. Simple regression analyses were done to assess the relationship between two parameters. A level of p < 0.05 was accepted as statistically significant.

Results

Age, increases in body weight before dialysis, and changes in body weight and serum concentrations of urea nitrogen and creatinine during dialysis did not significantly differ among the lower, middle, or higher BP groups (Table 1). Duration of hemodialysis was longer in the lower BP group than in the middle or higher BP groups. Changes in mean BP during dialysis were greater in the higher BP group than in the middle or lower BP groups. Heart rate did not significantly differ among the three groups.

Table 1. Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Lower BP</th>
<th>Middle BP</th>
<th>Higher BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (m, f)</td>
<td>16 (8, 8)</td>
<td>75 (37, 38)</td>
<td>23 (14, 9)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56±3</td>
<td>57±1</td>
<td>54±2</td>
</tr>
<tr>
<td>Duration of dialysis (months)</td>
<td>58±8*††</td>
<td>41±3</td>
<td>33±4</td>
</tr>
<tr>
<td>Increase in body weight before dialysis (kg)</td>
<td>2.1±0.3</td>
<td>2.1±0.2</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>Changes in body weight during dialysis (kg)</td>
<td>-2.0±0.3</td>
<td>-2.1±0.1</td>
<td>-2.4±0.1</td>
</tr>
<tr>
<td>Mean BP before dialysis (mmHg)</td>
<td>65±2**††</td>
<td>76.1**††</td>
<td>117±1**</td>
</tr>
<tr>
<td>Mean BP after dialysis (mmHg)</td>
<td>56±2**††</td>
<td>2.1**††</td>
<td>94±2**</td>
</tr>
<tr>
<td>Changes in mean BP during dialysis (mmHg)</td>
<td>-9±2**††</td>
<td>-16±1†</td>
<td>-23±2*</td>
</tr>
<tr>
<td>Heart rate before dialysis (beats/min)</td>
<td>82±3</td>
<td>80±2</td>
<td>82±2</td>
</tr>
<tr>
<td>Heart rate after dialysis (beats/min)</td>
<td>84±3</td>
<td>83±3</td>
<td>85±2</td>
</tr>
<tr>
<td>Serum urea nitrogen before dialysis (mmol/l)</td>
<td>26.9±4.7</td>
<td>28.3±4.3</td>
<td>26.5±5.1</td>
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<tr>
<td>Serum urea nitrogen after dialysis (mmol/l)</td>
<td>9.4±3.7</td>
<td>9.1±2.8</td>
<td>9.3±2.9</td>
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<tr>
<td>Serum creatinine before dialysis (mmol/l)</td>
<td>1.3±0.6</td>
<td>1.3±0.7</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>Serum creatinine after dialysis (mmol/l)</td>
<td>0.4±0.2</td>
<td>0.5±0.2</td>
<td>0.4±0.2</td>
</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; m, male; f, female. Lower BP group, a mean BP before dialysis less than 75 mmHg; middle BP group, a mean BP before dialysis of 76 to 109 mmHg; higher BP group, a mean BP before dialysis more than 110 mmHg. The causes of renal failure included chronic glomerulonephritis (n=70), diabetic nephropathy (n=40), hypertensive nephrosclerosis (n=2), and immunoglobulin A nephropathy (n=2). *p<0.05, **p<0.01 compared with the middle BP group, †p<0.05, ††p<0.01 compared with the higher BP group.

hHGF and BP

Before hemodialysis, the mean serum hHGF concentration was greater in the lower BP group (0.251±0.050 ng/ml, n=16) than in the middle (0.143±0.016 ng/ml, n=75) or higher (0.088±0.017 ng/ml, n=23) BP groups (Fig. 1A). After dialysis, the mean serum hHGF concentration increased compared with that before dialysis, and it was greater in the lower (1.854±0.242 ng/ml) than in the middle (1.280±0.120 ng/ml) or higher (0.688±0.103 ng/ml) BP groups (Fig. 1B). The increase in serum hHGF concentration during dialysis was greater (p<0.05) in the lower BP groups (+1.603±0.265 ng/ml) than in the middle (+1.137±0.110 ng/ml) or higher (+0.600±0.104 ng/ml) BP groups. Further, the increase in serum hHGF concentration during dialysis tended to be greater (p<0.1) in the lower (165-fold) than in the middle (42-fold) or higher (32-fold) BP groups. Serum hHGF concentration was inversely correlated with mean BP before dialysis (r=0.398, p=0.0001, n=114) and after dialysis (r=0.489, p=0.0001, n=114). Further, changes in serum hHGF concentration were correlated inversely with mean BP before and after dialysis (Fig. 2A, B). However, changes in mean BP during dialysis were not related with serum hHGF concentration either before dialysis (r=0.085, p=0.368, n=114) or after dialysis (r=0.002, p=0.983, n=114) or with changes in serum hHGF concentration during dialysis (r=0.011, p=0.911, n=114).

On the basis of our previous finding that the serum hHGF concentration may be different in diabetic patients and nondiabetic subjects (15), we divided the participants...
into diabetic and nondiabetic groups and investigated the relationship between serum hHGF concentration and mean BP in each group. In diabetic patients (n=40), the mean serum hHGF concentration before dialysis was greater (p<0.05) in the lower BP group (0.273 ± 0.070 ng/ml, n=11) than in the middle (0.145 ± 0.032 ng/ml, n = 21) or higher (0.107±0.026 ng/ml, n=8) BP groups. In nondiabetic subjects (n=74), the mean serum hHGF concentration before dialysis was greater (p<0.05) in the lower BP group (0.174 ± 0.032 ng/ml, n=27) than in the higher BP group (0.078 ± 0.022 ng/ml, n=15), but it was not greater than in the middle BP group (0.126 ± 0.016 ng/ml, n=32).

hHGF and NO

Mean plasma NO₃⁻ concentration before dialysis in the lower BP group (155.1 ± 15.9 μmol/l, n=16) was higher
was positively correlated with the serum hHGF concentration of IL-1 during hemodialysis (19). The concentration of IL-1 could peak in the post-dialysis period because the induction of IL-1 takes several hours to occur. In addition, it may take another several hours for IL-1 to stimulate hHGF or NO production (20). Thus, cytokines produced by dialysis treatment are not likely to affect hHGF or NO production during the same dialysis session, but plasma accumulation of cytokines such as IL-1 may contribute to the continued enhancement of production of hHGF or NO after dialysis. Further study is needed to determine whether circulating levels of IL-1 are greater in hemodialysis patients with higher serum hHGF or plasma NO$_3^-$ concentration than in those with lower concentrations.

Yang and colleagues reported that intravenous injection of hHGF produces a decrease in mean BP and an increase in heart rate in rats, and that these responses are significantly attenuated by intravenous pretreatment with NO synthase inhibitor (I2). This finding indicates a possible effect of circulating hHGF on NO release by affecting endothelial constitutive NO synthase. The strong and positive correlation between circulating concentrations of hHGF and NO$_3^-$ seen in this study may indicate that circulating hHGF is likely to be involved in the production and release of the endothelial constitutive type of NO in uremic patients. Serum concentration of hHGF after dialysis in the lower BP group is within the range previously shown to cause endothelial cell proliferation; concentrations of hHGF greater than 1.0 ng/ml are sufficient to stimulate DNA synthesis and proliferation in human aortic endothelial cells (8, 21). Repeated increases in serum hHGF induced by maintenance hemodialysis may elicit endothelial proliferation or repair of damaged endothelium, and increased or repaired endothelial cells may participate in enhancement of basal NO production. Further investigation is needed to determine whether changes in serum hHGF concentration seen in this study can elicit different responses in the production of constitutive type of NO in the endothelium of uremic patients.

The increased serum hHGF concentration in dialysis patients with chronic hypotension seen in this study is presumed not to result from low BP. Our study and another previous study (7, 22) have shown no relationship between serum hHGF concentration and hypotension. Nakamura et al. showed a positive correlation between serum hHGF concentration and systolic BP in essential hypertension (22, 23), and we found a weak positive correlation between serum hHGF concentration and BP in diabetic subjects (15). Although we cannot deny the possibility that continued hypotension can promote the production of hHGF via low perfusion or ischemia in organs, the inverse correlation between serum hHGF concentration and BP is likely to be peculiar to those subjects with end-stage renal disease undergoing maintenance hemodialysis.

The results of the present study indicate that circulating

Discussion

In the present study, the mean serum hHGF concentration before and after hemodialysis was greater in subjects with chronic hypotension than in those without, and the circulating hHGF concentration was inversely correlated with the mean BP before and after dialysis. In contrast, the serum hHGF concentration was not related to the changes in BP during dialysis. The plasma NO$_3^-$ concentration before dialysis, a stable index of endogenous NO production, was higher in subjects with chronic hypotension than those without, and it was correlated with the serum hHGF concentration before dialysis. These findings indicate that the genesis of chronic hypotension in hemodialysis patients may involve hHGF as well as NO.

hHGF has a strong affinity for heparin (6), which is routinely used in hemodialysis as an anticoagulant. Heparin-binding proteins like hHGF are reported to bind extracellular matrices such as heparan sulfate proteoglycans and to be stored as a complex with such molecules in vivo (16). Administration of heparin is thought to increase circulating hHGF concentration by modulating the mutual binding between hHGF and extracellular matrices (17). In a recent report (18), administration of 3,000 U or 10,000 U of heparin causes a 40- or 54-fold increase in plasma hHGF, respectively. In the present study, in which 3,000 U of heparin was administered during dialysis, the mean serum hHGF concentration after dialysis increased approximately 32- to 169-fold above the concentration before dialysis. These findings indicate that one explanation for the increase in serum hHGF concentration after hemodialysis is heparin administration. However, this increase in circulating hHGF concentration was not the same among the lower, middle, and higher BP groups; it was greater in the lower BP group than in the other groups. In hemodialysis patients with lower BP, the production and release into the circulation of hHGF may be enhanced and the amount of hHGF bound with extracellular matrices may be increased as compared with those patients with higher BP, although we do not have direct evidence to prove this hypothesis.

Cytokines such as IL-1 (11) are reported to promote hHGF synthesis as well as NO production. Bioincompatible membranes that activate complement, endotoxin fragments, or other bacterial toxins may stimulate the production of IL-1 during hemodialysis (19). The concentration
hHGF may be increased during hemodialysis in end-stage renal disease patients with chronic hypotension. Although there is a significant overlap between serum hHGF concentrations observed in subjects with lower or higher BP, hHGF is likely to be involved in chronic hypotension in hemodialysis patients, directly or indirectly, by affecting endogenous NO production. hHGF may be a new humoral factor that can contribute to solving the unknown mechanism of chronic hypotension associated with maintenance hemodialysis.

References