Hepatocyte Growth Factor and 24-Hour Ambulatory Blood Pressure Monitoring

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In recent years, many growth factors and cytokines have been shown to be related to arteriosclerosis, and hepatocyte growth factor (HGF) has been reported to be associated with hypertension. In the present study, we investigated the relationship between HGF and hypertension by measuring the serum HGF concentration and performing 24-h ambulatory blood pressure monitoring (ABPM) in 47 randomly selected male and female subjects who underwent a medical examination for cardiovascular disease. The results were as follows. 1) The mean serum HGF concentration in the subjects was 0.35 ± 0.14 ng/ml. 2) The serum HGF concentration was positively correlated with both the nighttime systolic and diastolic blood pressures \((r = 0.42, p < 0.05\) and \(r = 0.47, p < 0.01\), respectively). 3) No correlation was found between serum HGF concentration and daytime systolic or diastolic blood pressure. 4) When subjects were divided into two groups based on the difference between daytime and nighttime systolic blood pressure, i.e., a group in which the difference was less than 10 mmHg and a group in which the difference was 10 mmHg or more, the HGF concentration was significantly higher in the former group (0.39 ± 0.14 vs. 0.30 ± 0.12 ng/ml, \(p < 0.05\)); similarly, when subjects were divided into a group in which the difference between daytime and nighttime diastolic blood pressure was 5 mmHg and a group in which the difference was 5 mmHg or more, the HGF concentration was significantly higher in the former group (0.42 ± 0.15 vs. 0.31 ± 0.12 ng/ml, \(p < 0.05\)). The results indicated that there is a relationship between blood pressure measured by ABPM and serum HGF concentration, and that this relationship might be an index of damage to blood vessels in patients with hypertension.

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Key Words: hepatocyte growth factor (HGF), ambulatory blood pressure monitoring (ABPM), hypertension

Introduction

Hepatocyte growth factor (HGF) was first identified in the serum of partially (70%) hepatectomized rats, and has since been purified from rat platelets and the plasma of patients with fulminant hepatic failure \((1–3)\). Human HGF has been cloned and sequenced \((4, 5)\). HGF protein, which has been shown to consist of 728 amino acid, is inactivated by thrombin and kallikrein \((6–8)\). It has also been reported that HGF is produced by polymorphonuclear leukocytes \((9)\), vascular smooth muscle cells and endothelial cells in humans in vivo \((10)\).

Recently, this peptide has been shown to display not only mitogenic activity, such as that seen in liver regeneration, but also morphogenic, tumor suppressive and neovascularizing activities. It is known that the numbers of growth factors in the circulatory blood increase when complications in the brain, heart or kidneys arise in hypertensives as a result of damage to vascular endothelial cells \((11)\). Most of these growth factors act to stimulate growth of smooth muscle cells, but HGF acts specifically on the endothelium and does not promote the growth of smooth muscle cells \((12)\). Accordingly, HGF has recently been evaluated as a possible biochemical index of arteriosclerosis due to hypertension.

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fluctuation as determined by ABPM. The correlation between HGF concentration and blood pressure as a marker of hypertensive organ damage, we analyzed the consideration to be an index of arteriosclerosis in hypertension, study, in order to investigate the usefulness of HFG, which is considered to be an index of arteriosclerosis in hypertension, in which 24-h blood-pressure measurements were taken during free movement, have shown that there is a relationship between fluctuation in blood pressure and hypertensive organ damage and that there is a stronger correlation between daytime and nighttime systolic and diastolic blood pressures. Mean values of each difference were calculated (ΔSBP: dSBP - nSBP and ΔDBP: dDBP - nDBP, respectively.).

Subjects and Methods

Subjects
The subjects were selected from residents of Sobestu Town and residents of Tanno Town, a farming village, in Hokkaido, Japan. They underwent examinations for cardiovascular disease, including inquiries by the examining doctor, measurements of casual blood pressure in the sitting position, casual blood glucose level and HbA1c level, and other blood biochemical tests. Those who were being treated for and those who were afflicted by or were suspected of having diabetes, kidney disease or liver disease were excluded. A total of 47 people comprising 15 males (mean age, 62.6 ± 6.8 years) and 32 females (mean age, 59.7 ± 8.3 years) who were not taking any hypotensive drugs were selected as subjects. All subjects gave their informed written consent to participate in the study.

ABPM measurements were made at between 6:00 AM and 8:00 AM on the day after the start of ABPM recording. Casual blood pressure was measured twice, and the average of the two values was used for the analysis. Blood was collected from the median cubital vein. A casual blood glucose level of less than 126 mg/dl, an HbA1c value of less than 5.6%, and serum GOT and GPT of less than 50 U/l and 40 U/l, respectively, were considered normal.

Serum HGF Assay
HGF concentration was measured by enzyme-linked immunosorbent assay (24) (ELISA; Tokushu Immuno-Research, Tokyo, Japan) according to the following procedure. Fifty microliters of the sample was placed in each well of a 50 well microtiter plate with 50 µl of dilution solution, and the mixture was incubated for 1 h at room temperature. The solution was then discarded, and the wells were washed five times with 200 µl dilution solution. One hundred microliters of an enzyme-labeled monoclonal antibody of against HGF was then added to each well and incubated for 1 h at room temperature. After washing 5 times, 100 µl of an enzyme was added to each well and left for 30 min at room temperature. Finally, 50 µl of a reaction-termination solution was added to each well, and the absorption was measured twice for each sample and averaged. The resulting measurements ranged from 0.1 ng/ml to 3.0 ng/ml.

Statistical analyses were made using StatView software for Macintosh (5.1J). Values are expressed as the means ± SD. The relationships between blood pressure and other pa-
Parameters were investigated using multivariate analysis. Correlations between two variables were analyzed using Pearson’s correlation coefficient, and Student’s t-test was used for comparisons between groups. Values of $p < 0.05$ were considered to indicate statistical significance.

**Results**

The profiles of the 47 subjects are shown in Table 1. The mean HGF concentration in all subjects was $0.35 \pm 0.14$ ng/ml ($0.36 \pm 0.10$ ng/ml in males and $0.35 \pm 0.15$ ng/ml in females), with maximum and minimum values of 0.66 and 0.12 ng/ml, respectively. There was no significant difference between the HGF concentrations in males and females.

As shown in Fig. 1, no correlation was found between casual blood pressure and serum HGF concentration in either the systolic or diastolic phase.

The relationship between 24-h blood pressure levels and serum HGF concentrations is shown in Fig. 2. The values of dSBP, dDBP, nSBP and nDBP were 137 ± 15, 86 ± 11, 126 ± 17 and 79 ± 12 mmHg, respectively. Neither dSBP nor dDBP values were correlated with serum HGF concentration. However, serum HGF concentration was found to be positively correlated with nSBP ($r = 0.47$, $p < 0.01$) and nDBP ($r = 0.42$, $p < 0.01$).

![Fig. 1. The relationship between casual blood pressure and serum HGF concentration. No correlation was found between casual blood pressure and serum HGF concentration in either the systolic phase or diastolic phase.](image1)

![Fig. 2. The relationship between 24-h blood pressure levels and serum HGF concentrations. The values of dSBP, dDBP, nSBP and nDBP were 137 ± 15, 86 ± 11, 126 ± 17 and 79 ± 12 mmHg, respectively. The values of dSBP and dDBP were not correlated with serum HGF concentration. However, serum HGF concentration was found to be positively correlated with nSBP ($r = 0.47$, $p < 0.01$) and nDBP ($r = 0.42$, $p < 0.01$).](image2)
phase (Δ < 10 mmHg in 22 subjects and Δ ≥ 10 mmHg in 25 subjects) and diastolic phase (Δ < 5 mmHg in 17 subjects and Δ ≥ 5 mmHg in 30 subjects). In the systolic and diastolic phase, HGF concentration was significantly higher in the group with a smaller fall in blood pressure.

Table 2. Multiple Regression Analysis with HGF as the Dependent Variable

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standardized coefficient</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔDBP</td>
<td>- 0.06</td>
<td>- 0.387</td>
<td>- 2.316</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 0.0001</td>
<td>- 0.005</td>
<td>- 0.032</td>
</tr>
<tr>
<td>Gender</td>
<td>0.065</td>
<td>0.236</td>
<td>1.212</td>
</tr>
<tr>
<td>T. chol</td>
<td>&lt; 0.0001</td>
<td>0.149</td>
<td>0.876</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.068</td>
<td>0.346</td>
<td>1.668</td>
</tr>
<tr>
<td>nDBP</td>
<td>0.005</td>
<td>0.358</td>
<td>1.982</td>
</tr>
</tbody>
</table>

Dependent variable : HGF. $r^2 = 0.216$, $p < 0.05$. T. chol, total cholesterol.

It is well known that endothelial damage due to hypertension...
plays an important role in the progression to arteriosclerosis. Accompanying this progression is a decrease in vasodilator substances such as PGI2 and NO. Moreover, the actions of vasodilator substances containing many growth factors and cytokines have been reported to play a role in the repair process, and most of these growth factors and cytokines stimulate the proliferation of vascular smooth muscle cells. It is also known that there are some growth factors, such as vascular endothelial growth factor and HGF, which act on endothelial cells (11).

Although there have been several studies on the relationship between blood pressure and serum HGF concentration, only casual blood pressure has been investigated in these studies (13–17). However, it is known that there is a disparity between casual blood pressure measured at a medical examination and blood pressure measured at home. For this reason, home blood pressure is also important for the evaluation of possible complications and the prognosis of hypertension. In addition, interest has recently been shown in blood pressure fluctuation, particularly an excessive fall in night blood pressure [18, 25]. The sixth report by the JNC-VI (26) published in 1997 showed that ABPM is effective for the evaluation of the white-coat hypertension, drug-resistant hypertension, excessive fall in blood pressure due to hypotensive drugs, transient blood pressure rise, and autonomic nerve function. If, as mentioned previously, HGF is involved in the repair process of the endothelium, there might be a correlation between damage to the endothelium accompanying blood pressure rise and HGF concentration. In the present study, we therefore examined the possibility of using 24-h blood pressure and HGF concentration monitoring as indices of blood pressure and arteriosclerosis, respectively.

The results suggested that there is a close relationship between serum HGF concentration and nighttime blood pressure. The lack of correlation between HGF concentration and casual blood pressure in the present study was attributed to the disparity between casual blood pressure measured at a medical office and that measured at home i.e., the so-called white-coat phenomenon [18]. HGF concentration was significantly higher in the group in which there was only a small fall in nighttime blood pressure. Similarly, the lack of a clear relationship between daytime blood pressure and HGF concentration was attributed to the fact that many factors, such as movement and stress during daytime, have a greater effect on daytime blood pressure than on nighttime blood pressure.

In the present study, we found that HGF concentration was higher in the subjects who showed a smaller nighttime fall in either systolic or diastolic blood pressure. The same results were obtained when “dippers” and “non-dippers” were defined as subjects showing a fall in nighttime blood pressure of △ Đ 10% and △ < 10%, respectively, when the fall was calculated as △SBP/△SBP (27, 28). This result supports the finding of a previous report that damage to the vascular endothelium was greater and the degree of arteriosclerosis was more severe in “non-dippers” than in “dippers” (29, 30).

However, some unresolved issues remain. The first concerns the possible causal relationship between organ damage and dipper or non-dipper status. It has been pointed out that while an increase in blood pressure load due to a decrease in nighttime blood pressure fall is a cause of organ damage, it may also cause reduction or loss of blood pressure fluctuation due to maintenance of blood flow to the damaged organs, especially the brain (31). This possibility supports our speculation based on the present results that HGF increases to compensate for organ damage caused by loss of a nighttime fall in blood pressure. Previous reports have shown a decline in endothelial function in non-dipper hypertensives by plethysmography (32, 33).

The second issue is the reproducibility of ABPM. Since ABPM was only measured once in each subject in the present study, additional studies with larger cohorts will be needed to evaluate its reproducibility.

In order to investigate the effects of hypertension on damage to the endothelium, subjects with factors affecting the serum HGF concentration, such as liver damage, kidney damage and diabetes, were excluded from the analysis. It is known that diabetics have a lower serum HGF concentration than non-diabetics. This is because TGF-β, which inhibits the production of HGF, does not function under a high glucose condition, such as that in diabetes, and the cessation of the function of TGF-β in diabetes might play a major role in the progression of endothelial function disorder and arteriosclerosis (11, 34, 35).

Blood was collected from the median cubital vein in all of the subjects in the present study. A recent study showed that the tissue concentration of HGF in damaged blood vessels is significantly lower than that in normal blood vessels, suggesting that the decrease in HGF in damaged blood vessels may be a factor causing progression of blood vessel lesions, and that HGF in the circulating blood may increase to compensate for this effect (35). It is also thought that the reduction in clearance when the liver is damaged causes an increase in the concentration of HGF in the circulating blood (36).

Moreover, since it has been shown that the serum HGF concentration in hypertensive patients can be normalized by the administration of hypotensive drugs (14), it is possible that the serum HGF concentration could be used as an index of the curative effect as well as a parameter for the diagnoses and prognoses of organ diseases.

References
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