Malignant Hypertension: A Syndrome Accompanied by Plasmatic Diminution of Low and High Molecular Weight Kininogens

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SUMMARY Total kininogen (Kgn), kallikrein, and prekallikrein were measured in patients with malignant hypertension (MH), essential hypertension (EH), normotensive control (NC), and hypertensive and chronic renal failure (HRF). These components of the kallikrein-kinin system were related to the levels of creatinine and fibrinogen. High molecular weight Kgn and low molecular weight Kgn were also measured in blood samples from a peripheral vein, arterial blood, and suprahepatic vein in NC, EH, and MH. Results showed that total Kgn levels were diminished in MH and this diminution could not be ascribed to decreases in renal function, hematocrit, or fibrinogen levels. Appropriate antihypertensive treatment for over 1 year did not normalize Kgn levels in 10 of 11 patients. High molecular weight Kgn and low molecular weight Kgn were both diminished in MH (0.26 ± 0.04 nmol bradykinin/ml and 0.93 ± 0.12 nmol lysyl-bradykinin/ml, respectively) as compared to NC (0.39 ± 0.07 and 1.92 ± 0.16) and EH (0.51 ± 0.07 and 1.65 ± 0.13). Higher concentrations of high molecular weight Kgn were demonstrated in the suprahepatic vein as compared to arterial blood, demonstrating its synthesis by the liver. However, patients with MH had a diminished capacity to synthetize high molecular weight Kgn. A decrease in synthesis of high molecular weight Kgn may be a partial explanation for low levels of total Kgn. It is suggested that a lack of Kgn may play a role in the pathogenesis of MH. (Hypertension 5 (supp V): V-158-V-162, 1983)

KEY WORDS * malignant hypertension • kininogen • high molecular weight kininogen • low molecular weight kininogen

MALIGNANT hypertension (MH) is a syndrome of severe hypertension and several humoral alterations in vasopressor systems like the renin-angiotensin system,1 catecholamines,2 and, more recently, vasopressin.3 Furthermore, the kallikrein-kinin system has also been implicated in this disease in rats.4,5 and our group has demonstrated that kininogen (Kgn) is diminished in the plasma of patients with MH.6 Our observations were interpreted as reflecting either increased consumption or a diminished synthesis of Kgn.

This study was designed to explore our previous findings further by investigating the influence of renal function, hematocrit, plasma fibrinogen levels, and prolonged successful treatment of the malignant phase upon the levels of plasma Kgn in MH patients. Furthermore, both high molecular weight and low molecular weight Kgn were determined, to define the type of Kgn that is diminished in MH. Finally, an attempt to estimate the capacity of the liver to synthetize high molecular weight Kgn was also made.

Materials and Methods

Patients Studied

Study 1

In 10 normotensive controls, nine patients with mild-to-moderate essential hypertension (EH), 11 patients with end-stage renal failure and mild-to-moderate forms of hypertension (HRF), and 12 patients with MH, we determined the plasma levels of Kgn from a peripheral vein by incubation with trypsin and human urinary kallikrein.
Study 2

In seven patients with MH, 10 patients with mild-to-moderate EH, and eight normotensive controls (NC), we measured total plasma Kgn and low and high molecular weight Kgn from blood samples collected from a peripheral vein and other regions of the circulation (see protocols).

Patients with MH were selected from our emergency service; all had severe hypertension and Grade IV retinopathy. Patients with chronic renal failure were selected from our dialysis center, and in none was renal insufficiency due to arterial hypertension. Normotensive controls in Study 1 were healthy volunteers. In Study 2, normotensive subjects and patients with EH were selected from among patients undergoing arterial and venous catheterization because of suspected ischemic coronary disease or renovascular hypertension, respectively. Patients freely agreed to donate blood samples for this study.

Protocols

Blood samples (10 ml) from patients in Study 1 were collected from a peripheral vein following 1 hour of supine rest. In patients with chronic renal failure, blood samples were obtained immediately before dialysis. Blood pressure was also determined.

Blood samples from NC subjects and EH patients were collected during catheterization procedures from the following regions: a peripheral vein, suprahepatic vein, and aorta. Patients with MH were submitted to venous catheterization, and blood samples were collected from peripheral and suprahepatic veins. Arterial blood was collected from the radial artery through a direct needle puncture. Blood from the aorta and radial artery will be referred to as “arterial blood.”

Eleven patients with MH (four from the present study and seven from a previous one) were treated with several therapeutic regimens (diuretics, methyl-dopa, alpha- and beta-blockers, and captopril) to obtain adequate control of the arterial pressure. After 1 year of successful treatment, blood samples were again obtained.

Total Kgn, kallikrein, prekallikrein, hematocrit, and creatinine were determined only when samples obtained from the peripheral vein were available (Study 1). In those patients who also had samples collected from the suprahepatic vein and arteries (Study 2), high molecular weight and low molecular weight Kgn from blood samples were obtained, in addition to the same determinations made in the blood from the peripheral veins. Finally, in these Study 2 patients, peripheral venous fibrinogen levels were also determined.

Plasma Kininogens Determination

Kininogens were determined by bioassay from kinins generated in plasma after enzymatic degradation. Total Kgn was determined using either trypsin or human urinary kallikrein, a more specific kininogenase without bradykinin-potentiating effects. Human plasma kallikrein was employed for estimating high molecular weight Kgn, and low molecular weight Kgn was calculated by the difference between human urinary kallikrein and high molecular weight Kgn (Kgn-HuUK and HMW-Kgn).

Plasma samples, varying between 0.05 and 0.2 ml, were added to the guinea-pig ileum bath, and after 1 minute, excess of enzyme (human urinary or plasma kallikrein) was added. The reaction was recorded for 3 or 4 minutes. The contractions obtained were compared with standard curves of lysyl-bradykinin or bradykinin for determinations of human urinary kallikrein Kgn or high molecular weight Kgn.

Kallikreins

Plasma prekallikrein was converted to active kallikrein by prior incubation (for 4 minutes) with dextran-sulfate (0.5 mg/ml plasma). Both active and activated prekallikrein were estimated by their amidolytic activity upon the synthetic substrate H-D-Pro-Phe-paranitroanilide (Kabi Diagnostic Institute). In these experiments, 10 µl of plasma was incubated with 50 µl of substrate (204 µmol/ml incubate) in 0.05 M Tris HCl, pH 8, to a final volume of 1 ml for 4 minutes at 37°C.

Fibrinogen was determined by a radial immunodiffusion method (Böhring Institute). Plasma levels of creatinine were determined by the Jaffé reaction modified by Bartels and Bohmer. Blood pressure was determined by the auscultatory method using a mercury sphygmomanometer, and each value represents the mean of three consecutive determinations. Results are expressed as means ± se. Paired t test and analysis of variance were used when appropriate for statistical analysis.

Results

Mean age was not significantly different among groups studied (NC = 41 ± 4 years; EH = 43 ± 2 years; HRF = 33 ± 3 years; and MH = 45 ± 5 years). Both men and women were present in our groups (NC = 4 F, 4 M; EH = 5 F, 5 M; HRF = 6 F, 5 M; MH = 3 F, 4 M). Of 36 individuals studied, 23 were whites, three were racially mixed, and 10 were blacks. Patients with MH had higher (p < 0.01) mean arterial pressure (186 ± 6 mm Hg) and creatinine values (7.0 ± 2.0 mg/dl) when compared to NC (95 ± 2 mm Hg and 0.8 ± 0.1 mg/dl respectively) and EH patients (138 ± 4 mm Hg and 1.1 ± 0.1 mg/dl respectively). Values for hematocrit were lower in HRF patients (19.6% ± 1.5%) and highest for creatinine (12.3 ± 1.5 mg/dl, p < 0.01) as compared to other groups. MAP values were higher in patients with EH when compared to those of HRF and NC patients. No statistically significant differences were observed for the values of kallikrein, prekallikrein, and plasma fibrinogen levels (table 1).

As shown in figure 1, values of total Kgn determined by trypsin (Try) or human urinary kallikrein were diminished only in patients with MH (Kgn-Try = 2.53 ± 0.33 nmol bradykinin (BK)/ml; Kgn-HuUK = 1.45 ± 0.19 nmol lysyl-bradykinin (LBK)/
TABLE 1. Mean Arterial Pressure (MAP) and Biochemical Parameters of the Groups Studied

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hematocrit (vol%)</th>
<th>Creatinine (mg/dl)</th>
<th>MAP (mm Hg)</th>
<th>Fibrinogen (mg%)</th>
<th>Active kallikrein (nmol)</th>
<th>Activated prekallikrein (pNA/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (n = 8)</td>
<td>41.3 ± 0.9</td>
<td>0.8 ± 0.1</td>
<td>95 ± 2</td>
<td>300 ± 30</td>
<td>45.6 ± 21.2</td>
<td>372.2 ± 75.0</td>
</tr>
<tr>
<td>EH (n = 10)</td>
<td>42.6 ± 0.6</td>
<td>1.1 ± 0.1</td>
<td>138 ± 4†</td>
<td>310 ± 10</td>
<td>43.2 ± 7.7</td>
<td>473.3 ± 89.6</td>
</tr>
<tr>
<td>HRF (n = 11)</td>
<td>19.6 ± 1.3‡</td>
<td>12.3 ± 1.5‡</td>
<td>120 ± 5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MH (n = 7)</td>
<td>36.4 ± 2.0</td>
<td>7.0 ± 2.0*</td>
<td>186 ± 6*</td>
<td>310 ± 10</td>
<td>32.5 ± 8.8</td>
<td>446.5 ± 55.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. NC = normal control; EH = essential hypertension; HRF = hypertension renal failure; and MH = malignant hypertension.

*p < 0.01 (MH x EH and/or NC).

†p < 0.05 (EH x NC).

‡p < 0.05 (HRF x MH x NC, EH and MH).

ml, p < 0.05) as compared with NC (Kgn-Try = 7.82 ± 0.33 nmol BK/ml; Kgn-HuUK = 2.52 ± 0.13 nmol LBK/ml), EH (Kgn-Try = 6.98 ± 0.67 nmol BK/ml; Kgn-HuUK = 2.15 ± 0.15 nmol LBK/ml), and HRF (Kgn-Try = 7.24 ± 0.28 nmol BK/ml; Kgn-HuUK = 2.96 ± 0.13 nmol LBK/ml).

Figure 2 shows values of total Kgn, as determined by HuUK in eight normal controls, 19 patients with MH before treatment, and 11 after 1 year of successful treatment. After 1 year of treatment, Kgn values remained significantly lower in patients with MH. Only in one patient had Kgn levels increased to the normal range.

Figure 3 shows values of total Kgn, high molecular weight Kgn, and low molecular weight Kgn for MH, EH, and NC. Total Kgn values were significantly lower in patients with MH (1.19 ± 0.14 nmol LBK/ml; p < 0.05) than in patients with EH (2.15 ± 0.16 nmol LBK/ml) and in NC (2.31 ± 0.19 nmol LBK/ml).
Low molecular weight Kgn was also significantly lower in patients with MH (0.93 ± 0.12 nmol BK/ml; p < 0.05) when compared to patients with EH (1.65 ± 0.13 nmol BK/ml) and NC (1.92 ± 0.16 nmol BK/ml). Finally, high molecular weight Kgn was also significantly lower in MH (0.26 ± 0.04 nmol BK/ml, p < 0.05) than in EH (0.51 ± 0.07 nmol BK/ml) and in NC (0.39 ± 0.07 nmol BK/ml).

No significant correlation was found in patients with malignant hypertension between total Kgn or low molecular weight Kgn or high molecular weight Kgn and levels of creatinine or hematocrit. Values of total Kgn and low molecular weight Kgn determined in the suprahepatic vein, arterial blood (from aorta and radial arteries), and peripheral vein for NC, EH, and MH are shown in table 2. In MH patients, Kgn levels were always lower than that of those with EH and NC in all three vascular areas. High molecular weight Kgn showed a clear tendency to be higher in the suprahepatic vein than in the aorta and peripheral vein in NC, EH, and MH. This tendency was not statistically significant in patients with EH, but achieved statistical significance in MH (suprahepatic vein = 0.31 ± 0.04 nmol BK/ml > arterial = 0.23 ± 0.05 nmol BK/ml, p < 0.05) and in NC (suprahepatic vein = 0.59 ± 0.08 nmol BK/ml > arterial = 0.43 ± 0.07 nmol BK/ml > peripheral vein = 0.39 ± 0.07 nmol BK/ml; p < 0.05). Values of total Kgn and low molecular weight Kgn determined in the suprahepatic vein, arterial blood, and peripheral vein did not present a statistically significant difference in each of the groups studied (NC, EH, and MH).

**Discussion**

The kallikrein-kinin system (KKS) has been extensively studied in different types of hypertension. However, few studies have focused on plasma components of this system. We have previously demonstrated that the levels of kininogen are very low in the plasma of patients with malignant hypertension. The present observations confirm and extend this finding.

Patients within the various groups studied did not differ significantly with respect to age, sex, or ethnic group, a variable known to be associated with alteration in the KKS. As expected, patients with MH had the highest arterial pressure levels and also presented moderate-to-severe impairment in renal function. This latter alteration, however, cannot explain the observed diminution in plasma Kgn levels since patients with severe renal failure and hypertension had normal levels of Kgn. Thus, renal losses of low molecular weight Kgn, which has a molecular weight close to that of albumin, is not likely to explain the diminution of the total Kgn levels.

Plasma prekallikrein and kallikrein levels were kept within the normal range in MH, thus not favoring the hypothesis that increased activity of this enzyme could explain our data. Normal levels of fibrinogen in our patients with MH do not favor the hypothesis that activation of the coagulation system, known to occur in MH, could be consuming Kgn. More subtle alterations in the coagulation process, not reflected by alterations in plasma fibrinogen levels, cannot be ruled out by our data.

Appropriate medical treatment for over 1 year did not normalize the levels of Kgn except for one patient. This observation was seen earlier in our patients followed for 3 months. Since the normalization of arterial pressure was accomplished by several different therapeutic regimens, one cannot ascribe the lack of normalization of Kgn to a drug-induced phenomenon. Thus, the mechanism responsible for diminution in Kgn in MH is still operative after 1 year of blood pressure control.

It is known that Kgn in human plasma is present in two forms: low molecular weight Kgn (MW = 60,000) and high molecular weight Kgn (MW = 200,000). Since both low molecular weight and high

**Table 2. Total, High-, and Low Molecular Weight Kininogen (Kgn) for Normotensive Control (NC), Essential Hypertension (EH), and Malignant Hypertension (MH)**

<table>
<thead>
<tr>
<th>Kininogen value</th>
<th>Groups</th>
<th>Suprahepatic vein (SHV)</th>
<th>Arterial blood (A)</th>
<th>Periperal vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Kgn</td>
<td>NC (n = 8)</td>
<td>2.13 ± 0.26</td>
<td>2.14 ± 0.17</td>
<td>2.31 ± 0.19</td>
</tr>
<tr>
<td>Human urinary kallikrein</td>
<td>EH (n = 10)</td>
<td>2.18 ± 0.19</td>
<td>2.24 ± 0.16</td>
<td>2.15 ± 0.16</td>
</tr>
<tr>
<td>nmol lysyl-bradykinin/ml</td>
<td>MH (n = 7)</td>
<td>1.39 ± 0.18*</td>
<td>1.17 ± 0.11*</td>
<td>1.19 ± 0.14*</td>
</tr>
<tr>
<td>High molecular weight Kgn</td>
<td>NC (n = 8)</td>
<td>0.59 ± 0.08*</td>
<td>0.43 ± 0.07</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>nmol bradykinin/ml</td>
<td>EH (n = 10)</td>
<td>0.58 ± 0.10</td>
<td>0.47 ± 0.07</td>
<td>0.51 ± 0.07</td>
</tr>
<tr>
<td>MH (n = 7)</td>
<td>0.31 ± 0.04†</td>
<td>0.23 ± 0.05*</td>
<td>0.26 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>Low molecular weight Kgn</td>
<td>NC (n = 8)</td>
<td>1.54 ± 0.19</td>
<td>1.71 ± 0.12</td>
<td>1.92 ± 0.16</td>
</tr>
<tr>
<td>nmol lysyl-bradykinin/ml</td>
<td>EH (n = 10)</td>
<td>1.63 ± 0.15</td>
<td>1.77 ± 0.12</td>
<td>1.65 ± 0.13</td>
</tr>
<tr>
<td>MH (n = 7)</td>
<td>1.08 ± 0.16*</td>
<td>0.94 ± 0.11*</td>
<td>0.93 ± 0.12*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. 
*p < 0.05 (MH × EH and/or NC). 
†p < 0.05 (SHV × A). 
‡p < 0.05 (SHV × A and/or peripheral vein).
molecular weight Kgns are substrates for human urinary kallikrein, incubation with this enzyme results in generation of kinins that express the total amount of Kgn present in plasma. We thus decided to determine each form of Kgn by incubating plasma with human urinary kallikrein and with human plasma kallikrein. It is clear from our results that both high molecular weight and low molecular weight Kgns are similarly diminished in the plasma of MH patients. In these patients no statistically significant correlation was found between Kgn levels and plasma creatinine or hematocrit levels. Accordingly, neither the decreased renal function nor the low hematocrit explain low levels of Kgn.

One possible explanation for our data is that we could be dealing with a diminished synthesis of Kgn in MH. Accordingly, we decided to measure Kgn levels in venous blood from the liver, an organ thought to synthesize this substrate. From our data, it is evident that in both NC and MH subjects there was a statistically significant difference in the plasma high molecular weight Kgn when we compared values from the arterial blood and suprahepatic vein. A similar although not statistically significant difference was also observed in EH. Thus, these results are compatible with the theory that high molecular weight Kgn is added to arterial blood by the liver. Low molecular weight Kgn was not consistently higher in the suprahepatic vein. This is probably due to the fact that it may be synthesized in several glandular organs and not only in the liver.

High molecular weight Kgn levels were diminished, however, in all vascular territories studied. Thus, the gradient in the suprahepatic vein observed for MH was not sufficient to increase the circulating levels of high molecular weight Kgn. The total amount of high molecular weight Kgn synthesized by the liver in a given period of time is given by the suprahepatic-arterial difference for high molecular weight Kgn times the hepatic flow. Since this difference is lower in MH (0.31 - 0.23 = 0.08) than in NC (0.59 - 0.43 = 0.16) and since hepatic blood flow is not likely to be increased several times in MH, the amount of high molecular weight Kgn synthesized by the liver in MH is lower than that in NC. Thus, our patients may actually have a diminished capacity to synthesize high molecular weight Kgn. Whether this diminution in synthesis is linked to the malignant phase or is a hereditary trait cannot be answered by our data. This diminished synthesis, however, is not the only cause for the diminution of total Kgn since low molecular weight Kgn was also diminished in the plasma of patients with MH.

In summary, we extended our observations that total Kgn is diminished in MH. This diminution cannot be explained by decreased renal function, low hematocrit, or increased coagulation. Appropriate medical treatment for over 1 year did not normalize Kgn levels. Furthermore, total Kgn diminution is explained by decreases in high as well as low molecular weight Kgn. Finally, a decreased capacity to synthesize Kgn in MH is also suggested. The real meaning of these findings is not clear, but it is possible that a lack of appropriate amounts of Kgns may be related to the pathogenesis of malignant hypertension.

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Malignant hypertension: a syndrome accompanied by plasmatic diminution of low and high molecular weight kininogens.
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