Malignant Hypertension: A Syndrome Associated with Low Plasma Kininogen and Kinin Potentiating Factor

FERNANDO A. ALMEIDA, M.D., REGINA C. R. STELLA, M.D., ANITA VOOS, M.S., HORACIO AJZEN, M.D., AND ARTUR B. RIBEIRO, M.D.

SUMMARY Plasma levels of kininogen, kallikrein, and prekallikrein were determined in patients with malignant hypertension (MH) and compared to normotensive controls (NC) and patients with mild to moderate essential hypertension (EH). Also, a recently described kinin potentiating factor (KPF) was estimated by dividing the value of kininogen determined by trypsin (Kgn-Try) by that of kininogen determined by human urinary kallikrein (Kgn-HuUK). No significant alterations were detected among plasma values of prekallikrein and kallikrein of MH as compared to NC. However, Kgn-HuUK values were significantly lower in MH (1.9 ± 0.1 μgLBK/ml) as compared to EH and NC (2.7 ± 0.1 μgLBK/ml and 3.0 ± 0.2 μgLBK/ml respectively, p < 0.05). Furthermore, KPF values were also low (p < 0.05) in MH (1.6 ± 0.3) when compared with similar values obtained in EH and NC (3.0 ± 0.2 and 2.8 ± 0.1, respectively). Adequate control of blood pressure levels for 90 days in MH group caused no significant alterations in plasma levels of kininogen and KPF. It is suggested that diminished kininogen levels as well as a decrease in a kinin potentiation KPF that is generated in plasma by trypsin may be involved in the pathogenesis of human malignant hypertension.

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KEY WORDS • malignant hypertension • plasma kallikrein • kininogen • kinin potentiating factor

MALIGNANT hypertension is a syndrome in which several humoral alterations of vasopressor systems like renin-angiotensin system, catecholamines, and more recently vasopressin have been implicated in the pathogenesis of the vascular lesion. The kallikrein-kinin system has been implicated in several hypertensive states, since kinins are vasoconstrictors and also influence water and electrolyte excretion. A defective kallikrein-kinin system has been recently suggested in malignant, but not benign, hypertension in the rat.

In the present work we evaluated the levels of some plasma precursors of kallikrein-kinin system in malignant hypertension, compared to "benign" hypertension and normal subjects, by studying plasma levels of kininogen, kallikrein, and prekallikrein. Also, a newly described kinin potentiating factor was estimated in these subjects since it is known that several potentiating substances of both exogenous or endogenous origin may influence the activity of the kallikrein-kinin system.

Material and Methods

Patients and Controls

Twelve patients with malignant hypertension (MH), 16 with mild to moderate essential hypertension (EH), and 10 normal controls (NC) were studied. All patients with malignant hypertension, selected from our emergency facilities, had very high arterial blood pressure levels and grade 4 retinopathy. Three patients had essential MH, two had end stage renal failure, and the remainder had no workup to exclude renovascular hypertension or pheochromocytoma. Patients with mild to moderate EH were selected from our hypertension clinic and had no clinical or laboratory evidence of secondary hypertension. Normal controls were selected among staff members. All patients with MH were admitted to our University Hospital, and patients with mild to moderate EH were studied as outpatients. Both patients and normotensive controls agreed to donate blood samples for this study.

Experimental Protocol

Patients with MH had basal values of arterial pressure registered, blood samples collected, and were then treated with captopril (SQ 14,225, Squibb and Sons) with an initial dose of 150 mg/day. Blood pressure was taken every 15 minutes, and a second dose of captopril was repeated after 2 hours if diastolic pressure did not fall below 130 mm Hg. Furosemide was given after this second captopril dose when the...
diastolic pressure remained above 130 mm Hg. After this initial test, captopril was maintained in a daily dose of 150 mg every 8 hours in association with furosemide given in doses ranging from 40 to 120 mg/24 hours. In some patients, the addition of propranolol (120 to 240 mg/24 hrs) and/or prazosin (2 to 6 mg/24 hrs) was also necessary to obtain adequate control of blood pressure. After a variable period of time, necessary to control arterial pressure all patients were discharged from hospital and followed in our clinic. Routine determination of serum creatinine and plasma renin activity (PRA) were carried out for both hypertensive and control subjects.

Basal values of prekallikrein, kallikrein, and kininogen were measured and kinin potentiating factor (KPF) estimated from blood samples drawn from normal controls and from hypertensive subjects before treatment in the supine position for at least 60 minutes. To assess the influence of treatment upon kininogen and KPF, blood samples from supine MH patients were drawn at 4 hours on Days 1, 2, 7, 15, 30, 60 and 90 after the initiation of treatment. Mean arterial pressure (MAP) was calculated immediately before all blood sample collections.

### Total Plasma Kininogen

Total plasma kininogen was estimated by the amount of bradykinin liberated after incubation with trypsin or of lysyl-bradykinin liberated after incubation with a glandular kininogenase (human urinary kallikrein). Kinin Potentiation Factor (KPF) was defined as the relationship between the amount of bradykinin liberated by trypsin and the amount of lysyl-bradykinin liberated by glandular kininogenase from the same plasma sample, as previously described.

### Plasma Kallikrein

Plasma prekallikrein was converted to active kallikrein (1 min) with celite (1 mg/ml of plasma). Both active plasma kallikrein and activated prekallikrein were estimated by measuring their esterase activity upon p-tosyl-L-arginine [H]-methyl-ester (H-TAME). In these experiments, 20 µl of plasma was incubated with 10 µl of [3H] TAME (0.047 µCi/mole, Biochemical and Nuclear Corporation, Burbank, Georgia) in 30 µl of 0.08M Tris, pH 8.0, for 30 minutes at room temperature. A standard human kallikrein (0.450 esterase units) was used for the calibration curves. One esterase unit is equal to 1 µmole TAME hydrolyzed/min, at 37°C.14

PRA was determined in blood for at least 1 hour in the supine position, collected using a commercial angiotensin radioimmunoassay kit. Plasma levels of creatinine were determined by Jaffé reaction modified by Bartels and Bohmer. Blood pressure was determined by an auscultatory method using a mercury sphygmomanometer; each value represented a mean of three consecutive determinations. Results are expressed as mean ± se. Statistical analysis were done by paired t test or analysis of variance.

### Results

As seen in table 1, the patient groups were similar with regard to age and sex. Most subjects were white, 10 racially mixed, and one black. Patients with malignant hypertension showed higher MAP, PRA, and creatinine values compared to the other groups. Kallikrein and pre-kallikrein levels in patients with malignant hypertension (6.1 ± 1.1 mU and 50.4 ± 6.4 mU respectively) were not statistically different from control values (5.6 ± 1.1 mU and 49.6 ± 5.6 mU).

Figure 1 shows values of kininogen (Kgn) and kinin potentiating factor (KPF) for controls and untreated hypertensive groups. Kininogen levels determined by incubation with trypsin (Kgn-Try) were very similar in controls (8.3 ± 0.4 µgBRK/ml) and in patients with malignant hypertension (2.8 ± 0.5 µgBRK/ml) when compared to controls (8.3 ± 0.4 µgBRK/ml; EH = 2.7 ± 0.1 µgLBK/ml; and MH = 1.9 ± 0.3 µgLBK/ml). Kgn-Try levels were significantly lower in patients with malignant hypertension (2.8 ± 0.5 µgBRK/ml) when compared to both control and EH groups (p < 0.01). Similarly, low levels of kininogen were found in MH patients after incubation of human plasma with human urinary kallikrein (Kgn-HuUK) (NC = 3.0 ± 0.2 µg/LBK/ml; EH = 2.7 ± 0.1 µgLBK/ml; and MH = 1.9 ± 0.3 µgLBK/ml). Again, Kgn-HuUK was significantly lower in MH as compared to NC and EH groups (p < 0.01). However, the latter differences were less remarkable than those observed with Kgn-

### Table 1. Composition of the Groups Studied

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Ethnic group</th>
<th>Creatinine (mg%)</th>
<th>PRA (ng/ml/hr)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>32 ± 2</td>
<td>5</td>
<td>M</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>Essential hypertensive (n = 16)</td>
<td>39 ± 3</td>
<td>9</td>
<td>7</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>131 ± 3†</td>
</tr>
<tr>
<td>Malignant hypertensive (n = 12)</td>
<td>36 ± 3</td>
<td>3</td>
<td>9</td>
<td>4.0 ± 1.1*</td>
<td>12.5 ± 6.0*</td>
<td>171 ± 6*</td>
</tr>
</tbody>
</table>

F = female; M = male; W = white; M = mixed; B = black; PRA = plasma renin activity. MAP = mean arterial pressure.

*p < 0.01 (malignant hypertension vs essential hypertension and/or control).

†p < 0.05 (essential hypertension vs control).
Normal controls (n = 10)
Mild and moderate essential hypertension (n = 16)
Malignant hypertension (n = 12)

**FIGURE 1.** Values of kininogen, as determined by trypsin (Kgn-Try) or human urinary kallikrein (Kgn-HuUK), and of kinin potentiating factor (KPF).

Try. The KPF was of equal magnitude in control (2.8 ± 0.1) compared to EH (3.0 ± 0.2). In the MH group, KPF (1.6 ± 0.3) was lower than it was in other groups (p < 0.01). Hence, patients with malignant hypertension exhibited very low kininogen levels and KPF values.

Mean values for MAP, Kgn-Try, Kgn-HuUK, and KPF for nine patients with MH before and during 90 days of treatment are presented in figure 2. MAP showed a sharp initial fall (from 172 ± 6 to 121 ± 5 mm Hg) after only 4 hours of treatment and remained in the same range up to the 90th day. Values of Kgn-HuUK showed only minor fluctuations during all treatment period, thus remaining in the low range. Values of Kgn-Try were stable up to the 7th day of treatment and showed a small but sustained increase from the 15th day of treatment. However, values of Kgn-Try were not significantly different except for Day 15, which was higher than control (4.6 ± 0.5 μgBK/ml vs 2.8 ± 0.5 μgBK/ml, respectively, p < 0.05). Values of KPF showed a small but insignificant rise as of the 15th day, reflecting the observed increase in Kgn-Try. Thus, even with successful control of MAP in MH patients, no normalization of plasma kininogen levels was observed. Furthermore, KPF showed only a tendency to increase after the 2nd week of treatment.

**Discussion**

As would be expected, patients with MH in our series showed very high blood pressure levels, papilledema, increased PRA, and impaired renal function. An interesting finding was the low circulating kininogen levels associated with a diminished kinin potentiating factor. Diminished renal function is not likely to be responsible for these findings since our MH patients had a renal function ranging from normal to very low levels (plasma creatinine = 1.0 to 13.6 mg%). Furthermore, data from our laboratory have shown that in patients with renal failure and moderate hypertension, Kgn and KPF levels are within the normal range (unpublished observations). In patients with mild to moderate hypertension, levels of kininogen and kinin potentiating factor were similar to those of normal subjects.

In view of the fact that kininogen, as estimated by trypsin (a nonspecific protease), is much higher than that obtained by HuUK (a specific lysyl-bradykinin generator) it is reasonable to assume that in our conditions trypsin liberates peptides that are also capable of potentiating bradykinin action. These peptides were estimated by the ratio of the Kgn-Try/Kgn-HuUK, called kinin potentiating factor (KPF), and were strikingly decreased in patients with MH (fig. 1). Even with good control of blood pressure levels, no important variations in kininogen or KPF were observed for up to 90 days in patients with MH.

The real meaning of the very low circulating plasma kininogen levels, as estimated by HuUK in patients with malignant hypertension, is unclear. To explain this finding we could postulate a diminished synthesis
or an increased consumption or a combination of both. Our data do not favor increased activity in plasma kallikrein, since the values were similar in normals and MH subjects. However, an increased consumption of kininogen by other enzymes like glandular kallikrein or even those of the fibrinolytic system cannot be ruled out. Finally, the possibility of a diminished synthesis could also explain our findings but cannot be examined from our data. It is possible, however, that hepatic synthesis of kininogen could be impaired by vascular damage in the liver related to MH. Our data do not permit discrimination among the hypotheses described above.

It is known that a variety of agents can potentiate kinin action in vitro. In our study, a KPF generated by trypsin was diminished in the MH group but was present in equal amounts in normal and EH patients. Since we have previously demonstrated the peptide nature of the KPF, it is tempting to speculate that the diminution of this factor plays a role in malignant hypertension. It is interesting however, to observe that adequate control of the blood pressure levels of our MH patients was not accompanied by correction of kininogen and KPF levels. Whether this correction will eventually occur in these patients is still to be established in the future.

In summary, we have documented a diminution in kininogen plasma levels in malignant hypertensive patients in whom a kinin potentiating factor was also low. The real meaning of these findings is unclear, but it is tempting to speculate that impairment of the most potent natural vasodilator system, the kallikrein-kinin system, is involved in the pathogenesis of malignant hypertension.

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F A Almeida, R C Stella, A Voos, H Ajzen and A B Ribeiro

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