RENIN, ANGIOTENSIN II AND JUXTAGLOMERULAR APPARATUS IN LIVER CIRRHOSIS

Kazuoki Kondo, Ryuichi Nakamura, Ikuo Saito, Takao Saruta, and Shun Matsuki

It is now generally accepted that plasma renin and angiotensin are frequently increased in patients with cirrhosis and ascites!–7 and that aldosterone stimulated by the renin-angiotensin system plays an important role in the salt and water retention in this edema state. A lot of studies have been presented to explain this phenomenon. However, the precise cause of elevated plasma renin is not known and it remains to be determined what kind of role increased levels of renin and angiotensin are playing in cirrhosis. As it is well known that the liver is influencing upon several components of the renin-angiotensin system, such as renin substrate, converting enzyme, destruction of renin and angiotensin, and angiotensinase activity, simultaneous measurements of various components of the renin-angiotensin system are necessary to know the precise mechanism of this system in the liver disorders.

The present experiment was undertaken to evaluate more comprehensively the renin-angiotensin system in patients with chronic hepatitis and cirrhosis with or without ascites, by measuring plasma renin activity (PRA), plasma renin concentration (PRC), plasma renin substrate (PRS) and angiotensin II concentration (Ang II) simultaneously, and by observing juxtaglomerular apparatus (JGA) in a few autopsy cases.

SUBJECTS AND METHODS

Key Words:
Liver disorders
Renin-Angiotensin system
Juxtaglomerular cell

The subjects in this study consisted of 44 patients with cirrhosis and chronic hepatitis. The group comprised 33 males and 11 females, ranging in age from 32 to 80 years. Among them, 17 patients had chronic hepatitis, 17 patients had cirrhosis without ascites and 10 patients had cirrhosis with ascites. The diagnosis of cirrhosis was based on clinical findings, supported by laboratory tests, scintillation scanning of the liver, and when necessary (14 patients), it was confirmed by histological examination of hepatic tissues. Patients with chronic hepatitis had been treated more than one year, and had no signs and symptoms such as jaundice, splenomegaly, angioma, gynecostasia, testicular atrophy, palmar erythema, esophageal varices, abnormal BSP or ICG retention, and increased IgA globulin level. Tissue diagnosis of chronic hepatitis was established in 8 patients. The control group consisted of 10 subjects without evidence of specific diseases, ranging in age from 22 to 60 years (3 females and 7 males). Any patient received no treatment with diuretic agents and no salt restriction. Peripheral venous blood samples were taken from the patient supine in the morning, and were put into the tube containing 1 mg EDTA, 0.66 mg 8-hydroxyquinoline and 0.2 mg dimercaprol per ml of plasma to block the converting enzyme and angiotensinase.

PRA, PRC and PRS were determined by Skinner's method. Ang II was determined by radioimmunoassay kit using Gocke's method.

JGA was studied according to the method as previously reported. The tissues obtained at necropsy from 6 patients who died of cirrhosis,
or of cirrhosis with hepatoma were fixed in Bouin solution and embedded in Epon 812 by the method of Luft. The blocks were cut by the ultramicrotome in 0.5–1.0μ thick sections. After then the epoxy resin was removed by the method of Mayer and his associates. The sections were stained with the method of Bowie. The patients in whom JGA was observed were all males and had an average age of 58.7 (range 53 to 70). Five patients with ascites had been treated by sodium restriction (6 gm salt per day) for several weeks before death, and one patient without ascites had received no salt restriction. The control group consisted of 8 patients, rang-
Fig. 3. Plasma renin substrate in chronic hepatitis, liver cirrhosis without ascites and liver cirrhosis with ascites.
(Values represent means ± standard deviation)

Fig. 4. Angiotensin II concentration in chronic hepatitis, liver cirrhosis without ascites and liver cirrhosis with ascites.
(Values represent means ± standard deviation)

...ing in age from 24 to 70 years (5 males and 3 females) who died of traffic accidents or of acute pneumonitis, and had no salt restriction before death.

We evaluated the state of JGA by counting juxtaglomerular cells per glomerulus (JGCC/G), and by estimating the degree of granularity of JGA as juxtaglomerular granulation index (JGI) according to the method of Hartroft and Hartroft.16

RESULTS

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PRA in cirrhotic patients with ascites (4.0 ± 3.0 ng/ml/hr; mean ± SD) was significantly greater than that in normal subjects (1.5 ± 0.6 ng/ml/hr) (p < 0.05) (Fig. 1). Even in cirrhotic patients without ascites PRA (2.3 ± 0.9 ng/ml/hr) was also greater than that in normal subjects (p < 0.05). PRC was also higher in cirrhotic patients with ascites (10.6 ± 2.8 U/ml) than in normal subjects (6.5 ± 0.8 U/ml) (p < 0.01) (Fig. 2).

Although it was not statistically significant, the mean value of PRC was slightly increased in cirrhotic patients without ascites (7.2 ± 2.2 U/ml).

PRS, produced by the liver, seemed to decrease gradually with the progress of liver cirrhosis (Fig. 3). The mean value of PRS in liver cirrhosis without ascites was 739 ± 139 ng/ml and that in liver cirrhosis with ascites was...
Fig. 7. Hypergranulated juxtaglomerular cells in the case H. K. with liver cirrhosis with ascites. The glomerulus is located at the left upper part of the field. ×1000

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>BP mmHg</th>
<th>Na mEq/L</th>
<th>K mEq/L</th>
<th>Ascites</th>
<th>Urine Protein ng/ml/hr</th>
<th>PRA</th>
<th>JGI</th>
<th>JGCC/G</th>
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<tr>
<td>T. I.</td>
<td>70</td>
<td>male</td>
<td>120/70</td>
<td>135</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
<td>1.8</td>
<td>25.0</td>
<td>5.4</td>
</tr>
<tr>
<td>K. T.</td>
<td>55</td>
<td>male</td>
<td>102/64</td>
<td>123</td>
<td>4.0</td>
<td>+</td>
<td>-</td>
<td>1.6</td>
<td>36.8</td>
<td>7.4</td>
</tr>
<tr>
<td>M. K.</td>
<td>55</td>
<td>male</td>
<td>124/90</td>
<td>122</td>
<td>3.2</td>
<td>+</td>
<td>+</td>
<td>7.5</td>
<td>25.0</td>
<td>10.0</td>
</tr>
<tr>
<td>H. K.</td>
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<td>male</td>
<td>128/82</td>
<td>142</td>
<td>4.1</td>
<td>+</td>
<td>+</td>
<td>4.8</td>
<td>100.0</td>
<td>8.2</td>
</tr>
<tr>
<td>T. K.</td>
<td>53</td>
<td>male</td>
<td>120/80</td>
<td>134</td>
<td>3.4</td>
<td>+</td>
<td>+</td>
<td>12.0</td>
<td>30.7</td>
<td>5.5</td>
</tr>
<tr>
<td>M. K.</td>
<td>62</td>
<td>male</td>
<td>150/80</td>
<td>130</td>
<td>3.6</td>
<td>+</td>
<td>±</td>
<td>4.0</td>
<td>35.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Mean ± SD 59 ± 15 124±14 131 ± 7 ± 0.5 5.3 ± 3.6 ± 26.3 ± 1.6

Control 50 ± 17 131±18 138 ± 6 ± 0.3 1.7 ± 0.3 ± 7.7 ± 0.5

Abbreviations: BP = Blood pressure, Na = Serum Na concentration, K = Serum K concentration, PRA = Plasma renin activity, JGI = Juxtaglomerular granulation index, JGCC/G = Juxtaglomerular cell count per glomerulus.

509 ± 210 ng/ml. These values were significantly lower than that in normal subjects (880 ± 76 ng/ml) (p < 0.05).

The level of Ang II, which is considered to play the most important physiological role in the control of water and electrolyte balance

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and blood pressure among many components of the renin-angiotensin system, seems to depend on plasma renin, renin substrate, converting enzyme and antitensinase activity. Therefore, it is important to determine Ang II in liver disorders, in which many components of the renin-angiotensin system are affected. In this study Ang II was 200 ± 128 pg/ml in cirrhotic patients without ascites and 285 ± 156 pg/ml in cirrhotic patients with ascites; both were significantly higher than the control level (124 ± 68 pg/ml) (p < 0.05) (Fig.4). From the above mentioned findings, it is certain that the renin-angiotensin system is stimulated even in liver cirrhosis without ascites. Furthermore, it was demonstrated that there was a significant correlation between PRA and Ang. II (Fig. 5), even though it was supposed that several components of the renin-angiotensin system were affected in liver cirrhosis.

As it was demonstrated that the activity of the renin-angiotensin system is stimulated even in liver cirrhosis without ascites, the state of the renin-angiotensin system was studied in chronic hepatitis. In chronic hepatitis, PRC, PRS, PRA and Ang. II were 6.3 ± 1.5 U/ml, 776 ± 131 ng/ml, 1.8 ±0.7 ng/ml/hr and 163 ± 106 pg/ml respectively. These values were slightly higher than those in normal subjects, but the differences were not statistically significant (p > 0.05).

The cause of the increased levels of renin and angiotensin in liver cirrhosis is still unknown. Several investigators have suggested that the renal hemodynamic changes play an important role in renin release in liver cirrhosis. In this study renal blood flow was not determined, but the relationship between PRA and serum creatinine was studied in cirrhotic patients with or without ascites, and in thirteen patients (4 with ascites and 9 without ascites) the relationship between PRA and creatinine clearance was also studied. In addition, JGA was observed in 6 patients with liver cirrhosis.

The correlation coefficients between PRA and serum creatinine were 0.207 in cirrhotic patients with ascites and 0.258 in those without ascites (Fig.6). In thirteen patients in whom creatinine clearance was studied, the correlation coefficients between PRA and creatinine clearance was -0.12. These values were statistically not significant (p > 0.05).

The data on JGA in 6 patients who died of liver cirrhosis (5 patients with ascites and one patient without ascites) are summarized in Table I. The observation of JGA in these patients disclosed the significant increase in JGI (42.3 ± 26.3) in comparison with that in controls (8.2 ± 7.0) (Fig.7). In this study, JGCC/G in cirrhotic patients was 7.3 ± 1.6, which indicated the moderate proliferation of juxtaglomerular cells. The average of JGCC/G was 6.0 ± 0.5 in controls. JGI of one patient without ascites was also slightly higher than that in controls. There were 3 patients whose serum Na was less than 132 mEq/L.

**DISCUSSION**

Although a lot of studies have confirmed that the renin-angiotensin system is activated in various liver disorders, it is still controversial about the mechanism how the renin-angiotensin system is stimulated and what kind of role it is playing in liver disorders. It is well known that the liver has effects upon many components of the renin-angiotensin system. Renin substrate is produced in the liver and renin and angiotensin are thought to be metabolized by the liver. In addition, the converting enzyme, the peptidase that converts angiotensin I to angiotensin II and angiotensinase which metabolizes angiotensin are also influenced in liver disorders. Therefore, it is of necessity to study each component of the renin-angiotensin system in order to know the state of the renin-angiotensin system in liver disorders. So we measured PRC, PRA, PRS and Ang. II simultaneously in chronic hepatitis and liver cirrhosis, and in addition, juxtaglomerular apparatus was observed in a few patients.

As suggested by other investigators, the present study confirmed the increased activity of the renin-angiotensin system in liver cirrhosis, in spite of the reduction in PRS.

In addition, this study revealed that PRC, PRA and Ang. II tended to increase in the early stage of liver cirrhosis before ascites did appear.

In order to explain these increases of PRC, PRA and Ang. II, two mechanisms have been postulated. Heacox et al and Christlieb demonstrated that the liver is the major site of renin inactivation. In addition, Barnard et al and Wernier et al demonstrated the impaired hepatic inactivation of renin in cirrhosis, and postulated that the failure of the cirrhotic liver to perform renin inactivation may contribute to increased PRA.

The other explanation is that renin production by the kidney is increased in liver cir-
In order to prove the increased renin secretion by the kidney, it is necessary to study the JGA where renin is thought to be produced, because renin inactivation might change in this disorder as described above.

In this study we observed JGA in autopsy materials from 6 cirrhotic patients and proved moderate proliferation of juxtaglomerular cells and increased JGI as also noted by Hartroft, Fisher and Hellstrom and Gliedman et al. Though some of the patients in whom JGA was observed in this study had been treated by slight sodium restriction, these data support the concept that renin production by the kidney is increased in liver cirrhosis.

Reeves et al. reported that juxtaglomerular cell count was increased in cirrhotic patients with a low serum sodium concentration, which has been consistently demonstrated to produce augmented activity of the renin-angiotensin system. In this study three patients showed hyponatremia less than 132 mEq/L, but other three patients showed the normal levels of serum sodium. So it might be difficult to attribute the increased renin production by JGA in this disease to a low serum sodium concentration.

Brown et al. reported that PRA was at or near normal in cirrhotic patients without ascites, even though PRA was usually high in patients with ascites. Wolff et al. demonstrated that cirrhotic patients without ascites do not secrete excessive quantities of aldosterone which is believed to be controlled mainly by the renin-angiotensin system.

Our data, however, as noted by Imai and Sokabe showed moderate increase of PRA, PRC and Ang II in liver cirrhosis without ascites. Though Fisher and Hellstrom indicated that there were no significant difference in JGI between normal and cirrhotic patients without edema, in our experience JGA studied in one patient without ascites showed slightly increased JGI. This finding was correlative to the elevated plasma renin concentration, and activity obtained in cirrhotic patients without ascites. Gliedman et al., using the method of common bile duct ligation and division in dogs, also demonstrated increased JGI in dogs with ascites. Even in dogs without ascites, JGI was also increased when compared to normal dogs.

When the above findings are taken together, it is suggested that renin secretion by the kidney in cirrhotic patients is increased before the ascites develops. As one of the mechanisms for the increased production of renin in the kidney in the patients with cirrhosis, the renal hemodynamic changes induced by liver cirrhosis should be considered. It is widely accepted that renal perfusion is reduced in cirrhotic patients with functional renal failure and Baldus et al. found decreased renal blood flow even in the non-azotemic patients. The recent study by Kew and his colleagues postulated that the renal hemodynamic changes were also found in patients with liver cirrhosis without ascites. And there is a possibility that such hemodynamic instability stimulates renin secretion by the kidney. A significant correlation between renal and cortical blood flow and impairment of renal function as assessed by creatinine clearance was previously reported; and Schroeder et al. reported that there was a significant correlation between PRA and glomerular filtration rate in patients with cirrhosis and ascites. We studied the serum creatinine levels in cirrhotic patients, and in some of them creatinine clearance was also studied. In this study there was no correlation between PRA and serum creatinine levels not only in patients without ascites, but also in those with ascites. Even between PRA and creatinine clearance, we could not find a significant correlation. From these results, however, we cannot conclude that renal hemodynamic changes are not related to the hypersecretion of renin in these situations.

Angiotensin I and II directly stimulate adrenal cortex to secrete aldosterone which may play an important role in the development of ascites. Therefore, it is supposed that the increased renin and angiotensin in the non-ascitic stage of liver cirrhosis, regardless of the causes of renin stimulation, may contribute to the formation of ascites by increasing aldosterone, as suggested by other investigators.

Summary

The renin-angiotensin system in chronic hepatitis and liver cirrhosis was studied functionally and morphologically.

PRA, PRC and Ang II tended to increase in the non-ascitic stage of liver cirrhosis, and in the ascitic stage of liver cirrhosis those levels further increased, although PRS gradually decreased. Even in chronic hepatitis these values were slightly higher than control levels, but the differences were statistically not significant.

There was no significant relationship between serum creatinine levels and PRA. JGI was,
however, significantly increased in all 6 patients with liver cirrhosis, in whom JGA was observed. Even in a cirrhotic patient without ascites, JGI was slightly increased.

From these results it is concluded that the renin-angiotensin system is stimulated even in liver cirrhosis before ascites develops, and that the most parts of the increases in renin and angiotensin depend on renin production in the kidney.

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REFERENCES

20. LOYKE, H. F.: Experimental hypertension treated with 
37: 45, 1958.

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