Hypertensive Rats Produced by In Vivo Introduction of the Human Renin Gene

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We established an efficient and nontoxic in vivo gene transfer method mediated by the Sendai virus (hemagglutinating virus of Japan [HVJ]), liposomes, and nuclear protein. In this study, to produce a hypertensive model rat that is dependent on human renin, the human renin gene was introduced into adult rat liver by our efficient in vivo gene transfer method using HVJ and liposomes (HVJ-liposomes). The rats treated with HVJ-liposomes containing the human renin gene showed a significant elevation of blood pressure for 6 days compared with control rats, which received injections of HVJ-liposomes without the human renin gene. On day 5 after the transfer, human active renin as well as angiotensin II were found in the plasma of rats in which the human renin gene was introduced. Moreover, the blood pressure of these rats was significantly correlated with the plasma levels of human active renin and angiotensin II. To confirm that the elevated blood pressure was due to the expression of the human renin gene, we administered a newly developed specific human renin inhibitor, FK 906. The elevated blood pressure was normalized by the intravenous administration of this drug. These data indicate that this hypertensive rat was produced by the in vivo transfer of the human renin gene into rat liver and that the expressed human renin cleaved rat substrate (angiotensinogen). This hypertensive rat produced by in vivo gene transfer should be useful in further studies on hypertension. (Circ Res. 1993;73:898-905.)

KEY WORDS • renin • gene transfer • hemagglutinating virus of Japan • liposomes • hypertension

Un until recently, development of an animal model with a certain genetic disease has depended on classic substrain selection of a distinctive phenotype. However, this procedure is now being replaced by new biotechnological methods. One approach is construction of transgenic animals. Transgenic rats harboring the mouse Ren-2 gene and developing fulminant hypertension have been reported as a new model of human hypertension, although the active renin level in their serum is low. However, transgenic mouse harboring the rat renin gene did not show high blood pressure unless the rat angiotensinogen gene was expressed simultaneously. After introduction of the human renin gene, the transgenic mouse showed no hypertension. In general, transgenic technology is very complicated and time-consuming and still has limitations, such as low efficiency of technical success and unpredictable tissue-specific gene expression. Another approach is the direct in vivo gene transfer technique. Recently, we established a novel method for in vivo gene transfer using the Sendai virus (hemagglutinating virus of Japan [HVJ]), nuclear protein, and liposomes. This is a highly efficient technique with low toxicity and results in reliable gene expression in a target organ. Using this technique, we have succeeded in expressing the human hepatitis B virus surface antigen gene in the adult rat liver, thus providing an animal model of primate hepatitis. With this technique, we have also succeeded in expressing the human insulin gene in the adult rat liver and the simian virus 40 large T antigen gene in the adult rat kidney. The present study was conducted to determine whether the new technique is capable of introducing the human renin gene into the adult rat liver, thus providing a new hypertensive rat model dependent on human renin, which is a key enzyme of the renin-angiotensin cascade and plays a significant role in the pathogenesis of hypertension.

Materials and Methods

All studies were performed with the permission of the Ethical Committee of Animal Research, Osaka University Medical School.

Experimental Designs

Change in blood pressure after gene introduction. The first experiment was performed on 9 rats (5 experimental rats and 4 control rats) to measure blood pressure by direct monitoring through an intra-arterial catheter every day from 4 days before to 10 days after the gene transfer. On days 2 and 10, 2 mL whole blood was taken through the catheter from each rat, and plasma human active renin and angiotensin II (Ang II) were measured.

Endocrine examination in hypertensive and normoten sive rats. The second experiment was conducted with 10 rats (6 experimental rats and 4 control rats) to examine the correlation between systolic blood pressure and
human active renin and Ang II on day 5 after the gene transfer. In this experiment, blood pressure was measured by a tail-cuff method from 4 days before to 5 days after the gene transfer. On day 5, all rats were decapitated, and plasma human active renin and Ang II were measured.

Effect of human renin inhibitor in hypertensive rats. In the last experiment, a human-specific renin inhibitor, FK 906, was administered to confirm that the high blood pressure of the rats was dependent on the human renin, which came from the introduced human renin gene. Six experimental rats received this inhibitor. Three experimental rats received 25 mg/kg FK 906, and the other 3 rats received 2.5 mg/kg FK 906. In this third experiment, we also administered 25 mg/kg FK 906 to control rats (n=3), which received injections of HVJ and liposomes (HVJ-liposomes) without the human renin gene, spontaneously hypertensive rats (SHR, n=3), and two-kidney, one-clip (2KIC) hypertensive rats (n=3).

In these experiments, rats were considered hypertensive if their systolic blood pressure measured for 30 minutes at stable state was more than 140 mm Hg with direct blood pressure monitoring.

Construction of Plasmid

pUC-CAGGS (donated by Dr Junichi Miyazaki, Tokyo University), which contained the cytomegalovirus enhancer and the chicken β-actin promoter, was restricted with Xho I. Human renin cDNA (1.4 kb, donated by Dr Kazuo Murakami, Tsukuba University),16 isolated from PY118 by restriction with Pst I/Sac I, and ligated with Xho I linker, was cloned into this site to generate human renin CAGGS.

Cell Culture and Electroporation

Male Wistar rats (200 to 300 g) were anesthetized with pentobarbital (60 mg/kg) and used for preparation of isolated hepatocytes by in situ perfusion of the liver with Ca2+-free Hank’s solution and collagenase. He- patocytes were resuspended at a concentration of 4×106 to 10×106 cells/mL in William’s E medium (GIBCO, Grand Island, NY), pH 7.4, supplemented with 10% fetal calf serum. The cells were grown at 37°C in 95% air–5% CO2.

Approximately 107 hepatocytes were transfected with 100 μg of the plasmid (human renin CAGGS) by electroporation (Gene Pulser, Bio-Rad Laboratories, Richmond, Calif). We used electroporation for DNA transfection because of a report that DNA can be introduced into primary hepatocytes by electroporation but not with calcium phosphate precipitation or DEAE-dextran. When cells were suspended in Dulbecco’s phosphate-buffered saline, the highest transfection efficiency was achieved with a single voltage pulse of 250 to 300 V at 960 μF.

Preparation of HVJ-Liposomes

Lipids (phosphatidycholine, phosphatidylserine, and cholesterol) were mixed at a ratio of 4.8:1:2 (wt/wt/wt) as described previously. The lipid mixture (10 mg) in tetrahydrofuran was deposited in a rotary evaporator. Plasmid DNA was incubated with high-mobility group 1 (HMG 1), and this complex was shaken vigorously and sonicated to form liposomes containing plasmid DNA and HMG 1. The liposomes and HVJ, inactivated by ultraviolet irradiation (110 erg/mm2 per second) for 3 minutes just before use, were incubated at 4°C for 10 minutes and then at 37°C for 30 minutes with gentle shaking (2 strokes per second). This solution was centrifuged by sucrose density gradient. The top layer was collected for use.

Introduction of HVJ Liposomes Into the Rat Liver

Eight-week-old male Wistar rats received free access to normal rat chow (sodium, 260 mg/100 g; potassium, 750 mg/100 g) and tap water. Rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). The abdomen was opened with a median incision. Then a total volume of 4 mL of final HVJ-liposome suspension, which contained 10 mg lipids, 50 μg encapsulated DNA, and 64 μg HMG 1 with 1 mg/mL glucose and 1 mmol/L CaCl2, was injected under the perisplanchnic membrane of the liver. Control rats received injections of the same volume of HVJ-liposomes, but without the human renin gene, as the experimental rats.

Blood Pressure Measurement

Blood pressure was measured by the tail-cuff method or direct monitoring through an intra-arterial catheter. By the tail-cuff method, unanesthetized rats were warmed for several minutes in a box thermostatically controlled at 37° to 38°C and then introduced into a small holder for measurement of blood pressure. Blood pressure was also measured by inserting a catheter into the abdominal aorta via the right femoral artery of animals under ether anesthesia. After the operation, blood pressure was recorded in the conscious animals using a pressure transducer (model TP-400T), monitors (models AT-601G and AT-641G), and a recorder (model RTA-1300), all from Nihon Kohden Co, Tokyo, Japan. Systolic blood pressure was recorded continuously over a period of 15 minutes after attaining a stable state for more than 30 minutes. Blood pressure was measured by these methods every day at the same time of the day from 4 days before to 10 days after the gene transfer by direct monitoring and from 4 days before to 5 days after the gene transfer by a tail-cuff method.

Measurement of Human Renin

In the first in vivo experiment on days 2 and 10 after the gene transfer, blood samples were collected through an intra-arterial catheter. In the second in vivo experiment on day 5 after the gene transfer, blood samples were collected after decapitation. Samples of the medium of primary cultured hepatocytes were collected on day 2 after the gene transfer by electroporation. Blood and medium samples were collected into cooled tubes containing disodium EDTA (1 mg/mL) and centrifuged at 4°C. Human renin in plasma and medium was measured with a renin immunometric assay kit that used anti-human active renin monoclonal antibodies as described by Menard et al. This assay is highly specific for human active renin. We have found that this method can detect human active renin at 5 pg/mL, and the intra-assay and interassay coefficients of variation were 31% to 79% (n=19) and 5.0% to 9.8% (n=10), respectively, and the recovery of a known amount of renin was 91% to 122% (n=8). This direct and specific measurement of human renin used two pairs of monoclonal
antibodies (3E8 and 4G1) in an immunoradiometric assay. The specificity of each antibody for human renin was confirmed by the low percentage (<1%) of inhibition of rat renin enzymatic activity by the antibodies.  

Measurement of Angiotensins

Blood and medium samples were collected as previously described in “Measurement of Human Renin” above. The extractions of angiotensin I (Ang I) and II were obtained by high-performance liquid chromatography. First, medium and plasma samples were applied to an octyl minicolumn (Amprep C8, 500 mg, Amersham, Buckinghamshire, UK). After the column was washed with 0.1% trifluoroacetic acid (TFA), Ang II was eluted with 2 mL methanol/water/TFA (80:19.9:0.1 [vol/vol/vol]). The eluate was dried, the residue was resuspended in 100 µL of 0.1% TFA, and chromatography was performed using a Vydac C18 reversed-phase column (0.46×25 cm, Separations Group, Hesperia, Calif). The elution was carried out with an exponential gradient of methanol from 30% to 50% in 10 mmol/L sodium acetate buffer, pH 5.6, over a period of 15 minutes at a flow rate of 1 mL/min. In the appropriate fraction, samples were collected and dried in a vacuum centrifuge and redissolved in 0.1 mL. Tris acetate, pH 7.4, containing 2.6 mmol/L disodium EDTA, 1 mmol/L phenylmethysulfonyl fluoride, and 0.1% bovine serum albumin, pH 7.4. The sensitivity of this assay was 0.1 pg per tube. The cross-reactivity was 100% for angiotensin III and less than 0.1% for Ang I and rat atrial natriuretic factor. The concentrations of the extracted Ang I and II were measured by radioimmunoassay using specific anti-Ang I and anti-Ang II antibodies, respectively.

Administration of Renin Inhibitor

On day 5 before the gene transfer, we inserted a catheter into the abdominal aorta via the right femoral artery in ether-anesthetized rats. Then we measured blood pressure every day by direct monitoring. Systolic and diastolic blood pressures and heart rates were measured while the animals were at rest and freely moving. At the same time as the gene transfer, a catheter was inserted into the inferior vena cava. Through the catheter, three hypertensive rats were given 25 mg/kg FK 906, and the other three hypertensive rats received 2.5 mg/kg FK 906. Three normotensive control rats, which received HVJ-liposomes without the renin gene, three SHR, and three 2K1C hypertensive rats were also administered 25 mg/kg FK 906.

Human Renin Inhibitor FK 906

(2S,3S)-1-Cyclohexyl-3-hydroxy-6-methyl-2-[N-methyl-N-[2-(methyl-N-morpholinocarbonyl)amino]ethyl]aminocarbonyloxy]-3-phenyl-propionyl]-K-histidy[l]aminoheptane hydrochloride (FK 906) was synthesized by Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan; the molecular weight is 790.4. In the in vitro study, the IC50 value of FK 906 for human renin is 3×10−9 mol/L, and the value for rat renin is 1×10−5 mol/L.

Statistical Analysis

All values of blood pressure are expressed as mean±SEM. Two-way analysis of variance (ANOVA) was performed to assess the effects of gene transfer on the blood pressure with time, and one-way ANOVA was performed for analysis of data in Table 1. When differences were significant, Dunnett’s test was performed to assess the significance of differences of means from the basal blood pressure on the day of gene transfer. A value of P<.05 was considered significant.

Results

Before in vivo study, we examined the species specificity of the renin-angiotensin system, ie, the cross-reactivity of human renin and rat angiotensinogen. Two days after the transfer of the plasmid (human renin CAGGS) into cultured rat hepatocytes by electroporation, samples of medium were harvested for assays of human renin, Ang I, and Ang II. Hepatocytes in the dish produced and secreted human active renin and Ang I, which was converted to Ang II in the medium (Table 1). However, human active renin was not detected in any samples of the medium with control cells.

Table 1. Production of Angiotensin I and Angiotensin II by Rat Hepatocytes Transfected With the Human Renin Gene by Electroporation

<table>
<thead>
<tr>
<th>Renin Gene</th>
<th>Absent (n=8)</th>
<th>Present (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human active renin (pg/5×10^5 cells)</td>
<td>ND</td>
<td>808±55</td>
</tr>
<tr>
<td>Ang I</td>
<td>28±2</td>
<td>171±26*</td>
</tr>
<tr>
<td>Ang II</td>
<td>4.5±0.1</td>
<td>11.3±2.9†</td>
</tr>
</tbody>
</table>

ND indicates not detectable. Values are mean±SEM. *P<.01 and †P<.05 vs absence of renin gene.

Fig 1. Graph shows changes in systolic blood pressure of rats after in vivo gene transfer. Values are mean±SEM. The values of systolic blood pressure are obtained by a direct method using an indwelling arterial catheter for 10 days after the gene transfer. Experimental rats received injections of hemagglutinating virus of Japan (HVJ) and liposomes containing the human renin gene (●, n=4). Control rats received injections of HVJ and liposomes containing high-mobility group 1 alone without the human renin gene (○, n=5). In vivo gene transfer is indicated by the arrow. *P<.01 vs the level on day 0 (pretransfer level) by two-way analysis of variance followed by multiple comparison.
After the transfer of the human renin gene into the liver of the rats, significant increases were found from day 1 to day 6 in the systolic blood pressures of experimental rats compared with control rats.

Four days after the gene transfer, the systolic blood pressure reached a peak value of 144±2 mm Hg (n=4) by the direct method. This value was significantly higher than the value of 115±3 mm Hg (n=5) of control animals on the same day obtained by the direct method (P<.01). Moreover, on days 1, 2, 3, 5, and 6, significant increases were found in the systolic blood pressures of experimental rats compared with control rats. Thereafter, the blood pressure decreased to a level that was not significantly higher than that of the control rats on day 7 after the gene transfer. Rats treated with HVJ-liposomes without the human renin gene showed no increase in blood pressure (Fig 1).

In the second in vivo study, blood pressure was monitored by the tail-cuff method, and rats were decapitated on day 5 after the gene transfer. Four days after the transfer, the systolic blood pressure of experimental rats was 141±2 mm Hg (n=6) by the tail-cuff method. This value was significantly higher than the value of 115±2 mm Hg (n=4) in control rats on the same day obtained by the tail-cuff method. By this method, no increases were observed in the systolic blood pressure of control rats treated with HVJ-liposomes that did not contain the human renin gene (Fig 2a).

We then examined the correlation between the systolic blood pressure and plasma human active renin and plasma Ang II on day 5. A significant correlation (P<.05) was found between plasma human active renin concentration and systolic blood pressure in six experimental rats on day 5 after the transfer of the human renin gene (Fig 2b). Similarly, a significant correlation (P<.05) was also found between plasma Ang II concentration and systolic blood pressure (Fig 2c). The values show a significant correlation by simple linear regression analysis (y=1.9x+111, where y is systolic blood pressure and x is human active renin concentration in rat plasma [r=.95, P<.05, Fig 2b]), and y=0.05x+111, where y is systolic blood pressure and x is Ang II concentration in rat plasma [r=.95, P<.05, Fig 2c]).

On days 2 and 10 after the gene transfer in the first in vivo study and day 5 in the second in vivo study, we measured plasma human active renin and Ang II. As shown in Table 2, on day 5 the plasma levels of human active renin varied widely from 7.3 to 29.4 pg/mL, and plasma Ang II levels ranged between 230 and 1090 pg/mL in the same samples on the same day. On the other hand, human active renin was not detected at all in the plasma of control rats treated with HVJ-liposomes.
TABLE 2. Rat Plasma Levels of Human Active Renin and Angiotensin II

<table>
<thead>
<tr>
<th>Day</th>
<th>Active Renin (pg/mL)</th>
<th>Ang II (pg/mL)</th>
<th>Active Renin (pg/mL)</th>
<th>Ang II (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>ND</td>
<td>12.7</td>
<td>ND</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>14.8</td>
<td>ND</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>16.2</td>
<td>ND</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>12.4</td>
<td>ND</td>
<td>1.4</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>ND</td>
<td>14.0±0.9</td>
<td>4.1±2.5</td>
<td>167±93</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>10.2</td>
<td>ND</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>15.7</td>
<td>ND</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>12.1</td>
<td>ND</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>17.6</td>
<td>ND</td>
<td>10.1</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>ND</td>
<td>13.9±1.7</td>
<td>14.3±3.4*↓</td>
<td>550±127§</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>9.9</td>
<td>ND</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>17.2</td>
<td>ND</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>14.3</td>
<td>ND</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>18.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>ND</td>
<td>14.9±1.9</td>
<td>1.9±0.4</td>
<td>56±20</td>
</tr>
</tbody>
</table>

Ang II indicates angiotensin II; ND, not detectable.

*P<.05 vs active renin on day 2 in the presence of the renin gene; †P<.05 vs active renin on day 10 in the absence of the renin gene; ‡P<.05 vs Ang II on day 2 in the presence of the renin gene; §P<.01 vs Ang II on day 10 in the presence of the renin gene.

without the human renin gene. Plasma Ang II levels of control rats did not exceed those of the experimental groups on days 2, 5, and 10. In one experimental rat, higher levels of human active renin and Ang II were observed on day 2. On day 10, plasma human renin decreased, and in two of five rats, we could not detect human renin in plasma. The plasma Ang II level was slightly higher than that of control rats on day 10.

Finally, we examined the effect of the human renin inhibitor FK 906 on the hypertensive rats. As shown in Fig 3a, at day 5 after the introduction of human renin gene, the high blood pressure of the rats decreased to the normal (pretransfer) level by intravenous administration of 25 mg/kg FK 906. This typical recording shows a significant reduction in systolic blood pressure of 40 mm Hg and diastolic blood pressure of 20 mm Hg by FK 906 within a few minutes and recovery to the preadministration level in approximately 20 minutes. The mean±SEM decrease in blood pressure was 39.7±3.4/19.5±1.9 mm Hg (n=3). The hypotensive effect of FK 906 appears to be dose dependent, because injection of 2.5 mg/kg of this drug resulted in a slight decrease only in systolic blood pressure. Blood pressure changed from 144±2.4/102±1.9 to 136±3.2/101±2.1 mm Hg 5 minutes after administration (n=3). The blood pressure of control rats receiving 2.5 mg/kg FK 906 did not change before and after administration (before administration, 115±3.4/72±2.2 mm Hg; 5 minutes after administration, 112±2.9/75±1.4 mm Hg, n=3). A hypotensive dose (25 mg/kg) of FK 906 had no effect on blood pressures of normotensive control rats that received HVJ-liposomes without the human renin gene (Fig 3b), SHR (Fig 3c), or 2KIC hypertensive rats in which rat renin is responsible for the elevated blood pressure (Fig 3d).

In the combined studies, an HVJ-liposome suspension containing the human renin gene was injected into 19 rats, and we obtained 16 hypertensive rats that had higher blood pressure than pretransfer values before injections.

Furthermore, results of liver function tests showed no significant change after the gene transfer (plasma aspartate aminotransferase: control [before transfer], 56±4.4 IU/L; 5 days after the transfer, 57±3.2 IU/L; alanine aminotransferase: control [before transfer], 27±6.0 IU/L; 5 days after the transfer, 27±2.0 IU/L; total protein: control [before transfer], 6.3±0.1 g/dL; 5 days after the transfer, 5.7±0.2 g/dL; total cholesterol: control [before transfer], 48±4 mg/dL; 5 days after the transfer, 40±3 mg/dL).

**Discussion**

The efficiency of in vivo gene transfer depends on the method used for introduction of the foreign gene into
Fig 3. Recordings show the effect of human renin–specific inhibitor FK 906 (25 mg/kg) on the blood pressure and heart rate of hypertensive rats produced by in vivo transfer of the human renin gene. The effects of 25 mg/kg FK 906 on hemodynamics were observed in rats that received injections of hemagglutinating virus of Japan (HVJ) and liposomes with the human renin gene (a), in control rats that received injections of HVJ and liposomes without the human renin gene (b), in spontaneously hypertensive rats (c), and in two-kidney, one-clip hypertensive rats (d). Measurements of blood pressure and heart rate were performed by a direct method. The administration of the newly developed human renin inhibitor FK 906 into the inferior vena cava is indicated by the arrow.
the nuclei of target cells. In the present study, we could overcome this problem with the use of HVJ, liposomes, and nuclear protein HMG I. The contents of liposomes can be introduced into the cytoplasm 1000 to 10,000 times more efficiently by HVJ-liposomes than by liposomes alone without HVJ. Furthermore, introduction of foreign genes into the nuclei of target cells is 3 to 10 times higher on cotransduction with HMG I than with a foreign gene alone.

In the present study, with this efficient in vivo gene transfer method, we introduced the human renin gene into the adult rat liver. The present results support the conclusion that the elevated blood pressure resulted from the expression of the human renin gene introduced into the adult rat liver. Human renin generated by the introduced gene may yield Ang I from rat substrate in hepatocytes. This Ang I is then converted to Ang II, which is a hypertensinogenic hormone with vasoconstrictive and aldosterone-stimulating properties. However, recent reports suggest that human renin cleaves rat angiotensinogen very slowly. Infusion of human renin elevates blood pressure in nephrectomized and ganglion-blocked rats but does not do so in intact rats. Furthermore, the contribution of the intrinsic rat renin-angiotensin system to the produced hypertension in the present model is unclear. Probably, secretion of rat renal renin may be suppressed by a feedback mechanism due to the increased plasma Ang II generated by the human renin–rat angiotensinogen cascade. Thus, we consider that the main source of plasma Ang II may be the Ang I generated inside or in the immediate vicinity of rat hepatocytes, where generated human renin can react with rat angiotensinogen at high concentrations. If the cleavage of the rat substrate by human renin occurs inside the hepatocytes, produced Ang I should be secreted into the rat plasma, where it may be converted to Ang II. To support our hypothesis, we showed that not only human active renin but also a significant amount of Ang I and Ang II were produced in the medium of cultured rat hepatocytes into which the human renin gene (human renin CAGGS) had been transfected by electroporation (Table 1). In this study, the maximum observed level of plasma human active renin was 29.4 pg/mL, which is similar to the value in normotensive people. However, Ang II levels in experimental rats on day 5 were markedly higher than those of 2K1C hypertensive rats. These results may support our previously mentioned hypothesis that the reaction between human active renin and rat angiotensinogen occurred inside hepatocytes at high concentrations of both human active renin and rat angiotensinogen. Another explanation may be that secreted human renin was rapidly eliminated from the rat plasma. Nevertheless, the available in vivo data do not provide sufficient evidence for the location of the reaction between human renin and rat substrate. However, in any case, the kinetics of the exogenous and endogenous renin-angiotensin system is an interesting issue for further study.

In the present study, induced hypertension in the rat was transient, reflecting the change of plasma human renin. The data indicate that the maintenance of hypertension was limited by the cessation of the secretion of renin or Ang I from the liver through a constitutive pathway, the same as for other proteins produced in hepatocytes. We consider that the possible damage of hepatocytes by HVJ-liposomes, which may induce transient leakage of products, was negligible, judging from the normal values of liver function tests before and after gene transfer. We consider that the observed decrease in plasma human renin reflects the clearance of the exogenous gene from the target cells. As we reported previously, Southern blot analysis showed that the introduced foreign gene remained unchanged for at least 7 days but that it was rapidly eliminated within 12 days after the gene transfer. These results suggest that the secretion of human active renin into rat plasma decreased because the introduced human renin gene could exist in the target cells for at least 7 days and disappear within 12 days. These results also suggest that the foreign gene in hepatocytes was not integrated into the host genome. As a requisite for chronic study, a method for integration of exogenous DNA into chromosomal DNA using homologous recombination or autonomous replication of plasmid DNA in vivo should be developed.

In this study, we developed for the first time a new model of hypertension in the rat, direct in vivo introduction of the human renin gene, in which the introduced gene evoked a pathophysiological condition in the recipient animal. Although the observed hypertension was transient, this animal model should be useful in further studies of hypertension and the renin-angiotensin system, particularly in acute studies. If a longer duration of expression could be obtained, the technique would offer the benefit that foreign genes could be easily introduced and expressed in target cells and that model animals could be produced in a much shorter time and at higher efficiency compared with transgenic technology.

Our human renin–dependent rat model of hypertension should also be useful in the development of a human renin inhibitor. Indeed, studies of human renin inhibitors are a leading area of research, but so far only studies involving primates have been possible because of the high species specificity. With our system, rats can now be used for short-term studies of renin inhibitors specific for humans.

References
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