Kallikrein Gene Delivery Attenuates Hypertension and Cardiac Hypertrophy and Enhances Renal Function in Goldblatt Hypertensive Rats

Katsutoshi Yayama, Cindy Wang, Lee Chao, Julie Chao

Abstract—To demonstrate potential therapeutic effects of kallikrein gene delivery, we delivered adenovirus (Ad.CMV-cHK) carrying the human tissue kallikrein gene into two-kidney, one-clip Goldblatt hypertensive rats. A single intravenous injection of the recombinant adenovirus caused a delay of blood pressure increase that began 1 day after injection and continued for 24 days. A maximal blood pressure reduction was observed in rats receiving kallikrein gene delivery compared with control rats receiving Ad.CMV-LacZ (160±5 versus 186±7 mm Hg, n=6, P<.01). The expression of human tissue kallikrein mRNA was identified in the kidney, heart, aorta, and liver of rats receiving kallikrein gene delivery. Immunoreactive human kallikrein levels were measured in rat serum and urine in a time-dependent manner. Adenovirus-mediated kallikrein gene delivery caused a significant reduction in the left ventricular mass and cardiomyocyte size, as well as an increase in renal blood flow, urine flow, glomerular filtration rates, electrolyte output, and urine excretion. Enhanced renal responses were accompanied by significant increases in urinary kinin, nitrite/nitrate, and cyclic GMP levels. These findings show that the expression of human tissue kallikrein via gene delivery has protective effects against renovascular hypertension and cardiovascular and renal dysfunction. (Hypertension. 1998;31:1104-1110.)

Key Words: genetics • blood pressure • hypertrophy, cardiac • renal circulation • adenovirus

Tissue kallikrein (EC 3.4.21.35) is a serine proteinase that is capable of cleaving low-molecular-weight kininogen to produce the vasoactive kinin peptide.1,2 The binding of kinin with bradykinin B2 receptors produces a broad spectrum of biological effects, including smooth muscle contraction and relaxation, increase in vascular permeability, vasodilatation, electrolyte and glucose transport, and pain.2 Extensive clinical studies have shown that tissue kallikrein levels in urine are significantly reduced in patients with essential hypertension.3 Furthermore, a large family pedigree study has shown that a dominant allele expressed as high urinary kallikrein excretion may be associated with a decreased risk of essential hypertension.4 Because renal kallikrein originates from the kidney, these studies suggest that renal kallikrein defects may contribute to the development of human hypertensive diseases. In addition, reduced urinary kallikrein levels have also been observed in a number of genetically hypertensive rats.5,6 Together, these findings suggest that low renal kallikrein levels may contribute to hypertension and that high urinary kallikrein may offer a protective effect against the development of high blood pressure.

Intravenous infusion of purified tissue kallikrein or kinin into experimental animals caused a transient reduction of blood pressure. The blood pressure–lowering effect cannot be sustained because of the presence of tissue kallikrein inhibitors in the circulation and rapid cleavage of kinin peptides by degrading enzymes in the vasculature.5 Clinical studies have shown that the blood pressure of hypertensive patients can be temporarily lowered by oral administration of porcine pancreatic kallikrein.8 However, to achieve this hypotensive effect, repeated administration of purified tissue kallikrein is required, and the effect diminishes as soon as the treatment is terminated. By using molecular genetic approaches, we have demonstrated a direct link between alteration of tissue kallikrein gene expression and blood pressure regulation. We showed that transgenic mice overexpressing human tissue kallikrein under the control of the metallothionein metal response element or albumin gene enhancer/promoter are hypotensive throughout their life span.9,10 Administration of aprotinin, a tissue kallikrein inhibitor, or Hoe 140, a bradykinin B2 receptor antagonist, to these transgenic mice restored blood pressure to that of control animals. These results indicate that hypotension in kallikrein transgenic mice is mediated by binding of kinin to B2 receptors. This notion is further supported by the finding that transgenic mice overexpressing human bradykinin B2 receptor are hypotensive.11 These results provide direct molecular evidence linking the physiological function of the tissue KKS in blood pressure regulation. To further examine the effect of kallikrein gene...
expression on blood pressure regulation, we delivered the human tissue kallikrein gene into genetically hypertensive rats and found that somatic gene delivery of human tissue kallikrein caused a prolonged reduction of blood pressure for up to 8 weeks.12–15

In this study, we evaluated the effectiveness of tissue kallikrein gene delivery on blood pressure reduction in 2K1C Goldblatt hypertensive rats. In this experimental Goldblatt hypertensive model, the intrarenal RAS is upregulated during the initial phase of 2K1C hypertension. Plasma renin activity and angiotensin II levels were increased in 2K1C hypertensive rats.16,17 In contrast to the RAS, the expression of the renal kallikrein-kinin system (KKS) is increased during the initial phase but was downregulated in the chronic phase.18 It has been postulated that an imbalance in the activity of thevasopressor RAS versus the vasodepressor KKS may play an important role in the pathogenesis of hypertension after unilateral renal artery constriction. To investigate the role of the KKS in renovascular hypertension, the human tissue kallikrein gene was delivered via intravenous injection into 2K1C Goldblatt hypertensive rats. Adenovirus-mediated delivery of the human tissue kallikrein gene was shown to cause sustained delay of blood pressure increase for more than 24 days, attenuation of cardiac hypertrophy, and increased renal function. These results show that kallikrein gene delivery produces a wide spectrum of beneficial effects, making it an excellent candidate in treating renovascular hypertensive and cardiovascular diseases.

Methods

Preparation of Replication-Deficient Adenoviral Vector Ad.CMV-cCHK

Plasmid CMV-cHK was constructed as previously described.14 The expression of human tissue kallikrein cDNA, flanking the entire coding sequence, was under the control of the cytomegalovirus enhancer/promoter and by the bovine growth hormone gene polyadenylation signal sequence. Plasmid pAd.CMV-chK was constructed by inserting the Nael/NruI released fragment of CMV-cHK into the adenoviral shuttle vector pAd.Lnk.1 at an EcoRV site. The pAd.CMV-cHK plasmid DNA was purified using a Qiagen plasmid DNA kit, and the purified DNA was sent to the Institute for Human Gene Therapy, Wistar Institute, for generation of adenovirus Ad.CMV-cHK harboring the CMV-cHK-polyA transcription unit. Adenovirus harboring the LacZ gene under the control of the cytomegalovirus enhancer/promoter (Ad.CMV-LacZ) was obtained from the Institute for Human Gene Therapy, Wistar Institute, Philadelphia, Pa.

Animal Treatment

Goldblatt 2K1C hypertension was induced in 5-week-old male Wistar rats obtained from Harlan Sprague-Dawley (Indianapolis, Ind). The rats were prepared by placing a constricting silver clip (internal gap, 0.2 mm) around the left renal artery while rats were under sodium pentobarbital (50 mg/kg IP) anesthesia. One week after the surgery, rats were injected with 4×10^11 plaque-forming units of adenoviral particles of Ad.CMV-cHK or Ad.CMV-LacZ via the tail vein. All procedures complied with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Resources, National Academy of Sciences, Bethesda, Md).

Systolic Blood Pressure

Systolic blood pressure was measured with a photoelectric tail-cuff device (Natsume Co). This device requires minimal warming of rats (usually <15 minutes) before blood pressure determination and a brief period of restraint in a plastic cage. For each animal, the systolic blood pressure was represented as the mean of eight recordings.

RT-PCR Southern Blot Analysis of Human Tissue Kallikrein mRNA

Total RNA was extracted from fresh rat tissues by guanidine isothiocyanate.10 RT-PCR Southern blot analysis specific for human tissue kallikrein mRNA (5′ primer, 5′-CATTTCAGCACCTTTCA-3′; 3′ primer, 5′-GCCAAGGGGACGTAGC-3′; probe, 5′-ACGA CTTTCACAGCGTC-3′) was performed as previously described.13

Serum and Urine Collection

At various time points after injection of adenoviral vectors, 0.8 mL of blood was withdrawn from the tail vein. After coagulation, about 0.4 mL of serum was prepared by centrifugation. Serum was collected and subjected to the measurement of human tissue kallikrein and cGMP levels. The 24-hour urine samples were collected in metabolic cages at 14, 21, and 28 days after injection of adenoviral vectors. Urinary Na+ and K+ excretion was measured by flame photometry.

ELISA Specific for Human Tissue Kallikrein

The levels of human tissue kallikrein in serum or urine were determined by ELISA.12 The standard for human tissue kallikrein ranges from 0.4 to 25 ng/mL. Because the antibody recognizes only the active kallikrein, the immunoreactive kallikrein levels determined by ELISA represent active kallikrein.

Urinary NOx Measurement

Urine samples were sent to the New York Medical College for measurement of NOx content. Urinary NOx contents were measured by a colorimetric assay based on the Griess reaction.20

Radioimmunoassay for Kinin and cGMP

Urinary kinin levels were measured by a direct kinin radioimmunoassay as previously described.21 The procedure for assay of cGMP was conducted according to a previously described procedure.22

Measurement of Urine Flow Rate, GFR, and Renal Blood Flow

Adenovirus carrying the human tissue kallikrein was injected intravenously at 7 days after clipping of the left renal artery. The studies on renal function were performed at 14 days after injection of the vector or at 21 days after clipping of the left renal artery. Rats were anesthetized with pentobarbital (50 mg/kg IP) and placed on a heating pad for maintenance of body temperature at 37°C. After tracheotomy, a cannula was placed in the jugular vein for infusion of fluids and drugs. A cannula was placed in the right femoral artery for the measurement of blood pressure and for blood sampling. The bladder was cannulated to allow urine collection from the right kidney. The left kidney was exposed by a flank incision, freed of
perirenal tissue, placed in a Lucite cup, and bathed in 0.9% NaCl, and the ureter was cannulated. Hydropenic preparations were maintained by an intravenous injection of 1.2 mL of 0.9% NaCl containing 10% polyfructosan (Inutest, Laevosan) and 2% paraaminohippuric acid (PAH; Merck, Sharp & Dohme) via the cannula in the jugular vein during the experimental period. Forty-five minutes was allowed for the preparation to reach a steady state. Timed urine collections were obtained, with blood (0.6 mL) collected between pairs of clearance periods. For maintenance of hematocrit level, red blood cells from each blood sample were reconstituted to the same volume with 0.9% NaCl and reinjected through the arterial cannula. At the end of each experiment, kidneys were excised, blotted, and weighed. Urine volume was determined gravimetrically. Polyfructosan and PAH concentrations were determined by modified anthrone and colorimetric methods, respectively.

Heart and Left Ventricular Weight
At the conclusion of the renal function study, the heart was removed, and the atrium was carefully cut away and weighed. The right ventricular free wall was carefully dissected from the left. The intraventricular septum was thus included in the left ventricular weight.

Cardiomyocyte Diameter
Sections of the heart were preserved in 4% buffered formaldehyde solution and embedded in paraffin. Sections were cut to 5-mm thickness and stained with hematoxylin-eosin and analyzed microscopically and morphometrically. Cardiac myocyte diameters were measured in two perpendicular directions using an ocular micrometer with an engraved measuring scale. The ocular micrometer was calibrated against a stage micrometer, and conversion factors were calculated for low (>4 objective) and high (>45 objective) magnifications. Cardiac myocytes were judged to be cut in cross section when the shorter measurement was not more than 2 μm wider than the longer measurement. The average of the two measurements was then recorded as the cross-sectional diameter of the measured myocyte. The mean diameter of 200 cardiomyocytes in each group was measured with a calibrated eyepiece at a magnification of ×450. All sections were evaluated in a blind study in which knowledge of the group to which the measurements corresponded was revealed only after the data were tabulated.

Statistical Analysis
The results are expressed as mean±SEM for 5 or 6 animals. The statistical significance of the difference in systolic blood pressure between control receiving Ad.CMV-LacZ and rats receiving Ad.CMV-cHK was determined by ANOVA. In addition, we used an unpaired Student’s t test to assess the difference of physiological parameters between Ad.CMV-cHK and Ad.CMV-LacZ groups after kallikrein gene delivery.

Results

Intravenous Delivery of Human Tissue Kallikrein Gene Reduces Systolic Blood Pressure of 2K1C Goldblatt Hypertensive Rats
Fig 1 shows the effect of kallikrein gene delivery on systolic blood pressure of 2K1C Goldblatt hypertensive rats receiving adenoviral vectors Ad.CMV-cHK or Ad.CMV-LacZ. The systolic blood pressure of rats at 1 week after renal artery constriction was significantly higher (158±4 mm Hg, n=6, P<.01) than that of sham-operated rats (127±4 mm Hg, n=6). A single intravenous injection of the recombinant adenovirus harboring the human tissue kallikrein gene caused a delay of blood pressure increase that began 1 day after injection and continued for 24 days. A maximal blood pressure reduction of 26 mm Hg was observed 13 days after kallikrein gene delivery, compared with that of control rats injected with Ad.CMV-LacZ (160±5 versus 186±7 mm Hg, n=6, P<.01). At 31 days after gene delivery, there was no significant difference in systolic blood pressure of 2K1C rats receiving Ad.CMV-cHK (186±4 mm Hg, n=6) and Ad.CMV-LacZ (187±4 mm Hg, n=6).

Expression of Human Tissue Kallikrein mRNA in 2K1C Goldblatt Hypertensive Rats
Human tissue kallikrein mRNA in 2K1C Goldblatt hypertensive rats after gene delivery was analyzed with RT-PCR followed by Southern blot analysis using specific oligonucleotide probes for human tissue kallikrein. Total RNAs were prepared from heart, aorta, kidney, adrenal gland, and liver at 14 days after intravenous injection of adenoviral vectors Ad.CMV-chK or Ad.CMV-LacZ. Human tissue kallikrein mRNA was detected mainly in the liver and kidney and to a lesser extent in the heart and aorta (Fig 2, upper panel). The expression of human tissue kallikrein mRNA was not detected in control rats receiving adenoviral vector Ad.CMV-LacZ (Fig 2, upper panel). Similar levels of β-actin mRNA were detected in tissues of both experimental and control groups, indicating the integrity of RNA in these samples (Fig 2, lower panel). The results show that human tissue kallikrein is expressed in tissues relevant to cardiovascular and renal function after gene transfer in Goldblatt hypertensive rats.
Time Course of Immunoreactive Human Tissue Kallikrein in 2K1C Goldblatt Hypertensive Rats

Immunoreactive human tissue kallikrein levels in rats receiving human tissue kallikrein gene delivery were measured by an ELISA specific for human tissue kallikrein. Human tissue kallikrein in serum and urine of rats receiving kallikrein gene delivery displayed parallelism to the human tissue kallikrein standard curve, indicating their immunological identity. Human tissue kallikrein was not detected in the serum or urine of control rats receiving Ad.CMV-LacZ adenovirus (△).

Physiological Analysis of 2K1C Goldblatt Hypertensive Rats After Human Tissue Kallikrein Gene Delivery

The Table shows the results of physiological analysis of Goldblatt hypertensive rats 14 days after gene delivery. No apparent changes in the body weight and heart rate were observed in rats injected with Ad.CMV-chK compared with control rats receiving Ad.CMV-LacZ. Significant increases in urine volume (15.2±0.3 mL/d, n=6, \(P<.01\)) and water intake (38±6 mL/d, n=6, \(P<.01\)) were observed in rats receiving Ad.CMV-chK compared with control rats receiving Ad.CMV-LacZ. In 2K1C rats receiving Ad.CMV-chK, urinary sodium and potassium outputs were significantly increased compared with control rats (sodium, 232±21 versus 90±9 \(\mu\)mol/100 g body wt per day, \(P<.01\); potassium, 521±35 mEq/L).

Effects of Kallikrein Gene Delivery on Physiological Parameters in Goldblatt Hypertensive Rats

The Table shows the results of physiological analysis of Goldblatt hypertensive rats 14 days after gene delivery. No apparent changes in the body weight and heart rate were observed in rats injected with Ad.CMV-chK compared with control rats receiving Ad.CMV-LacZ. Significant increases in urine volume (15.2±0.3 mL/d, n=6, \(P<.01\)) and water intake (38±6 mL/d, n=6, \(P<.01\)) were observed in rats receiving Ad.CMV-chK compared with control rats receiving Ad.CMV-LacZ. In 2K1C rats receiving Ad.CMV-chK, urinary sodium and potassium outputs were significantly increased compared with control rats (sodium, 232±21 versus 90±9 \(\mu\)mol/100 g body wt per day, \(P<.01\); potassium, 521±35 mEq/L).

Values for each group are mean±SEM (n=4 to 6).

\(\ast P<.05\), Ad.CMV-chK vs Ad.CMV-LacZ; † \(P<.01\), Ad.CMV-chK vs Ad.CMV-LacZ. Statistical significance between the two groups was determined by unpaired Student’s \(t\) test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ad.CMV-LacZ</th>
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<tr>
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<tr>
<td>Heart rate, bpm</td>
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<td>433±25†</td>
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<td>Body weight, g</td>
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<td>Water intake, mL/d</td>
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<td>38±6†</td>
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<td>521±35*</td>
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<td>Creatinine, mg·100 g body wt(^{-1}·d^{-1})</td>
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<td>3.1±0.2</td>
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</tbody>
</table>

Figure 4. Time course of human tissue kallikrein levels in the serum (A) and urine (B) in 2K1C Goldblatt hypertensive rats after adenovirus-mediated kallikrein gene delivery. The levels of recombinant human tissue kallikrein in rats receiving Ad.CMV-chK injection were measured by a specific ELISA. n.d. indicates not detected (n=4).

Figure 5. Levels of kinins, NOx, and cGMP in urine of 2K1C Goldblatt hypertensive rats at 14 days after adenovirus-mediated kallikrein gene delivery. Ad.CMV-LacZ indicates 2K1C Goldblatt hypertensive rats receiving control adenovirus carrying the LacZ gene; Ad.CMV-chK, 2K1C Goldblatt hypertensive rats receiving adenovirus carrying the human tissue kallikrein gene.
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Increased Urinary Kinin, NOx, and cGMP Levels in Rats Receiving Kallikrein Gene Delivery

Fig 5 shows urinary kinin, NOx, and cGMP levels in 2K1C Goldblatt hypertensive rats at 14 days after gene delivery. Urinary kinin levels increased 10-fold after kallikrein gene delivery compared with those in control rats receiving Ad.CMV-LacZ (71.5 ± 10.6 versus 7.01 ± 0.3 ng/100 g body wt per day, n = 5, P < .01). Urinary NOx content increased 11-fold after kallikrein gene delivery compared with that in control rats (1.12 ± 0.07 versus 0.10 ± 0.02 μmol/100 g body wt per day, n = 4, P < .01). Urinary cGMP levels increased 1.8-fold after kallikrein gene delivery compared with those in control rats (30.4 ± 4.2 versus 16.6 ± 1.7 nmol/100 g body wt per day, n = 5, P < .01).

Protective Effects of Kallikrein Gene Delivery on Cardiac Hypertrophy in Goldblatt Hypertensive Rats

As shown in Fig 6A, mean left ventricular weight of the 2K1C group receiving Ad.CMV-LacZ (0.94 ± 0.03 g, n = 4) increased significantly compared with that of sham-operated rats (0.71 ± 0.06 g, n = 4, P < .01) at 14 days after gene delivery. Kallikrein gene delivery in 2K1C rats significantly reduced left ventricular weights compared with 2K1C rats receiving Ad.CMV-LacZ (0.67 ± 0.06 versus 0.94 ± 0.03 g, n = 4, P < .01). Similarly, mean heart weight of the 2K1C group receiving adenovirus Ad.CMV-LacZ (1.24 ± 0.04 g, n = 4) significantly increased compared with that of sham-operated rats (0.96 ± 0.01 g, n = 4), and kallikrein gene delivery significantly reduced heart weights compared with those in 2K1C rats receiving Ad.CMV-LacZ (0.96 ± 0.01 versus 1.24 ± 0.04 g, n = 4) (Table). Urinary creatinine excretion was similar between the two groups.

Effects of Kallikrein Gene Delivery on Renal Function in Goldblatt Hypertensive Rats

Fig 7 shows the results of renal hemodynamics in 2K1C Goldblatt hypertensive rats at 14 days after gene delivery. Kallikrein gene delivery caused a significant increase in urine flow rate (11.7 ± 0.6 versus 8.5 ± 0.3 mL/min/wt kidney wt⁻¹, n = 3, P < .01), GFR (1.19 ± 0.01 versus 0.83 ± 0.04 mL/min/wt kidney wt⁻¹, n = 3, P < .01), and renal blood flow (5.64 ± 0.04 versus 4.27 ± 0.15 μL/min/wt kidney wt⁻¹, n = 3, P < .01) compared with the group receiving the control adenovirus Ad.CMV-LacZ. In addition, GFR in 2K1C rats receiving control adenovirus Ad.CMV-LacZ significantly decreased (n = 3, P < .01) compared with that in sham-operated rats. Kallikrein gene delivery significantly increased GFR in Ad.CMV-chK rats (n = 3, P < .05) compared with sham-operated rats. Similarly, renal blood flow was significantly increased in Ad.CMV-chK rats (n = 3, P < .01) and was unchanged in Ad.CMV-LacZ rats compared with sham-operated rats.

Discussion

This is the first study to demonstrate that adenovirus-mediated kallikrein delivery is capable of delaying the development of renovascular hypertension in 2K1C Goldblatt hypertensive rats. We showed that a single intravenous injection of the adenovirus carrying the human tissue kallikrein gene into 2K1C hypertensive rats caused a rapid and profound effect on the progression of high blood pressure within 24 hours and the effect lasted for more than 24 days. In addition, these results clearly demonstrated that somatic delivery of the human tissue kallikrein gene could attenuate the development of renovascular hypertension.
of cardiac hypertrophy and alter renal hemodynamics in this experimentally induced hypertensive rat model. These findings suggest that kallikrein gene delivery has protective effects against renovascular hypertension and cardiovascular and renal dysfunction.

Goldblatt 2K1C hypertension represents an animal model of human renovascular hypertension in which renal artery stenosis is induced by placing a constricting clip around the left renal artery to partially reduce perfusion. Renovascular hypertension is characterized by increased levels of RAS components. Dramatic increases in blood pressure of the renovascular hypertensive Goldblatt rats were accompanied by elevated circulatory renin activity and angiotensin II concentrations that occurred in the period immediately following constriction of the renal artery. Short-term treatment with ACE inhibitor increased kinin excretion from the nonclipped kidney. ACE inhibition not only reduces angiotensin II formation but also augments local accumulation of kinins. It is possible that the altered balance in angiotensin-induced vasoconstriction and kinin-mediated vasodilation was responsible, at least in part, for the effects of systemic delivery of kallikrein gene on renal hemodynamics. Recently, it has been shown that endogenous kinins contribute to the increased renal function induced by ACE inhibitors in the nonclipped kidney of 2K1C hypertensive rats via bradykinin B₂ receptors. These findings suggest that kinins may exert a protective role in the action of ACE inhibitors in blood pressure homeostasis and cardiovascular and renal function. In addition, a previous study using bradykinin antagonists suggests that endogenous kinins contribute to the regulation of renal blood flow in the kidney. In this study, we show that urinary kinin levels markedly increased after kallikrein gene delivery, indicating a dramatic increase in intrarenal kinin levels in 2K1C hypertensive rats after gene transfer. These observations suggest that the enhanced renal function induced by kallikrein gene delivery was mediated by increased kinin levels.

Reduced urinary kallikrein levels have been documented in experimental hypertensive rats and essential hypertensive patients. It has been shown that renal kallikrein gene expression and urinary kallikrein excretion from the nonclipped kidney are maintained at levels not different from those of normal kidney. However, tissue kallikrein level is significantly lower in the clipped kidney than in the normal kidney. Furthermore, long-term renovascular hypertension significantly reduces bradykinin-induced endothelium-dependent relaxation. Consistent with these studies, we found that renal kallikrein mRNA and urinary kallikrein levels were reduced in 2K1C Goldblatt hypertensive rats (C. Wang, L. Chao, J. Chao, unpublished results, 1997). Collectively, these findings suggest that reduced activity of the renal KKS may contribute to the development of hypertension in 2K1C hypertensive rats.

Goldblatt 2K1C hypertension is associated with structural changes within the heart and blood vessels. Left ventricular hypertrophy has been demonstrated within 7 days of renal artery constriction in the rat, and vascular changes occur within 3 weeks. These structural alterations are an important factor in the maintenance of the elevated peripheral resistance seen in this model of hypertension. A nondepressor dose of an ACE inhibitor has been shown to prevent the development of cardiac hypertrophy in renal hypertension, and this protective effect was abolished by bradykinin B₂ receptor antagonist. Kinin accumulation resulting from kallikrein gene delivery may contribute significantly to the prevention of cardiovascular structural changes in 2K1C Goldblatt hypertensive rats. Although the expression level of human kallikrein mRNA in the heart of 2K1C rats was low, high levels of human kallikrein were detected in the bloodstream. Therefore, circulating or local tissue kallikrein/kinin might play a role in reversing cardiac hypertrophy in 2K1C hypertensive rats after kallikrein gene delivery.

The mechanisms of blood pressure reduction and increased renal function after kallikrein gene delivery appear to be mediated via kinin through an NOx-cGMP signal transduction pathway. It is well known that bradykinin can produce vasodilatory and natriuretic effects when infused into the renal artery. In addition, various kininase inhibitors are able to increase urinary kinins, sodium and water excretion, and papillary blood flow. Previously, we reported that systemic or local delivery of human tissue kallikrein in spontaneously hypertensive rats using naked plasmid DNA vector could cause a prolonged delay in blood pressure increase. The hypotensive effect of kallikrein gene delivery was abolished by in catibant (Hoe 140), a specific bradykinin B₂ receptor antagonist, suggesting that the blood pressure-lowering effect is mediated by the bradykinin B₂ receptor. Human tissue kallikrein and its mRNA can be identified in rat kidney and urine after kallikrein gene delivery. However, cellular localization of human tissue kallikrein in rat kidney has yet to be identified. The expression of human tissue kallikrein in 2K1C rats after kallikrein gene delivery could lead to increased kinin formation in the kidney. Activation of renal bradykinin B₂ receptors was indicated by increased urinary NOx and cGMP levels in 2K1C rats receiving kallikrein gene delivery. These findings suggest that binding of kinins to the B₂ receptor triggers the release of nitric oxide, which may subsequently activate cGMP. The influence of other potential second messengers such as eicosanoids in triggering the effects of kinin on blood pressure remains to be elucidated.

Adenoviral vectors have been used successfully to infect and express recombinant proteins in many tissues in a number of experimental animal models. When adenovirus encoding luciferase driven by the cytomegalovirus promoter was administered via intravenous injection, a strong transduction was observed in the liver, kidney, and spleen. The expression pattern of the human tissue kallikrein gene in the rat receiving adenovirus-mediated gene transfer via the intravenous route is similar to that of endogenous tissue kallikrein, except that rat tissue kallikrein is not expressed in the liver. In this study, we identified expression of recombinant human tissue kallikrein in tissues relevant to cardiovascular and renal function after intravenous injection of the replication-deficient adenovirus carrying the human tissue kallikrein gene. The route of delivery ensures a high level of immunoreactive kallikrein in the plasma, which was synthesized in the liver and secreted into the circulation. In addition, human tissue kallikrein was detected in rat urine at 1, 2, and 3 weeks after injection. The results suggest that recombinant human tissue kallikrein is synthesized locally in the kidney and cleared through the kidney in 2K1C hypertensive rats. It is possible
that human tissue kallikrein detected in rat urine could originate from the kidney as well as from the circulation.

Although adenovirus-mediated gene delivery results in high efficiency expression, the duration of the blood pressure–lowering effect is somewhat shorter than that observed with naked kallikrein DNA delivery. Because adenovirus does not integrate into the host genome, the injected DNA is eventually degraded in the cells. In addition to transient recombinant gene expression, adenovirus-mediated gene transfer also contributes to the problems of inflammation, and thus difficulties with vector readministration. These problems could be circumvented in part by coadministration of the vector with a cytotoxic and immune-suppressive drug such as cyclophosphamide. Cyclophosphamide can prevent the formation of neutralizing antibodies and stabilize expression of the transgene, thereby diminishing inflammation and prolonging transgene expression.

Recently, second-generation adenoviral vector has been shown to produce prolonged transgene expression and markedly reduce inflammation. Therefore, the development of future-generation adenoviral vector could potentially be used for studying the role of the human tissue kallikrein in cardiovascular and renal functions.

**Acknowledgments**

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**References**

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