Endothelial Function Predicts the Development of Renal Damage after Combined Nephrectomy and Myocardial Infarction

Peter Ochodnicky, Dick de Zeeuw, Robert H. Henning, C. Alex Kluppel, and Richard P.E. van Dokkum

Department of Clinical Pharmacology, University Medical Center Groningen, Groningen University Institute for Drug Evaluation, University of Groningen, Groningen, The Netherlands

It was demonstrated that individual renal endothelial dilatory function of the healthy rat predicts susceptibility to subsequent renal damage induced by 5/6 nephrectomy. In addition, it is reported that myocardial infarction (MI) that was performed upon unilateral nephrectomy (UNx) induced highly variable renal damage. Therefore, whether the variability in renal damage after MI could be explained by the variation in individual renal endothelial function before the induction of injury was studied.

Endothelium-dependent relaxation to acetylcholine was investigated in vitro in small arteries that were isolated from the extirpated kidney at UNx. MI was induced 1 wk after UNx by ligation of the left coronary artery. Proteinuria and systolic BP were evaluated weekly for 16 wk thereafter using metabolic cages and the tail-cuff method, respectively. Upon termination of the study, focal glomerulosclerosis was evaluated by histology as an additional marker of renal damage. After MI, nephrectomized male Wistar rats \((n = 15)\) gradually developed variable proteinuria, ranging from 20 to 507 mg/24 h at week 16, with an average systolic BP of 131 ± 7 mmHg. The individual renal endothelial function of the healthy rats predicted the extent of renal damage in terms of proteinuria \(r = -0.62, P = 0.008\) and focal glomerulosclerosis \(r = -0.70, P = 0.003\). The individual level of renal endothelial function in the healthy rat is able to predict the severity of renal damage that is induced by MI.

Further exploration of the underlying mechanisms may lead to discovery of preventive renoprotective therapies.


The susceptibility of the individual to develop proteinuria and subsequent renal damage shows large variability. Some individuals develop renal damage, whereas others do not. This interindividual variability cannot be explained by variation in BP levels \((1)\) or by differences in the degree of injury, such as subtotal nephrectomy \((2)\). Therefore, the variation in susceptibility has been proposed to be intrinsic to the kidney itself \((3)\). Indeed, we previously demonstrated individual differences in renal endothelial function of the healthy animal to predict severity of renal impairment after subtotal \((5/6)\) nephrectomy \((4)\).

Recently, we reported that myocardial infarction \((MI)\) leads to the development of proteinuria in the rat after unilateral nephrectomy \((UNx)\) \((5)\). Although the large infarcts induced more pronounced proteinuria than the small ones, proteinuria still varied to a great extent among the rats with relatively uniform MI sizes.

Given the above, we hypothesized that the variation in renal endothelial function among healthy animals predicts their susceptibility to develop proteinuric renal damage after UNx + MI. We used the rat model of cardiorenal interaction, in which MI is performed after UNx. In healthy kidneys that were removed at nephrectomy, endothelial function of small renal arteries was investigated. Subsequently, this baseline endothelial function was related to markers of renal damage \((proteinuria and glomerulosclerosis)\) that were measured in the remaining kidney 16 wk after MI.

Materials and Methods

Experimental Animals

Animal experimentation was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of the University of Groningen. Male Wistar rats \((250 to 275 g, n = 15; Harlan, Zeist, The Netherlands)\) were housed under standard conditions. Rats underwent surgical procedures for UNx \((followed by in vitro measurements of renal endothelial function)\) and MI. Subsequently, the animals were followed for 16 wk. Then the left kidney and the heart were removed under anesthesia, weighed, and analyzed for the markers of organ damage.

Surgical Procedures

UNx. Rats underwent UNx of the right kidney by laparotomy and under anesthesia with isoflurane 3% in \(N_2O/O_2\) \((1:2)\). The removed kidney was weighed and put into cold Krebs solution, and one small renal (interlobar) artery per kidney was prepared immediately for measurement of renal endothelial function in vitro.

MI. One week after nephrectomy, rats were intubated, ventilated (Amsterdam Infant Ventilator; Hoek/Loos, Schiedam, The Nether-
lands), and anesthetized by the administration of isoflurane 3% in N₂O/O₂ (1:2). MI was induced by ligation of the left ascending coronary artery as described previously (5). Variation in the infarct sizes was limited by the standard localization of the suture around the left ascending coronary artery. Subsequently, the wound was closed and anesthetics were replaced by 100% oxygen for a short while until the rat was able to breathe sufficiently on its own.

In Vitro Measurements of Renal Endothelial Function

Endothelial function of isolated small renal (interlobar) arteries was investigated in an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT) as described previously (4) and measured as endothelium-dependent relaxation to cumulative doses of acetylcholine (Ach; 10⁻⁸ to 10⁻⁴ mol/L) in the vessels that were preconstricted to 45 to 50% by phenylephrine (3 × 10⁻⁷ to 10⁻⁶ mol/L). We previously established that endothelial function measured in this way does not differ between arteries of left and right kidney of the same animal (4).

Markers of the Renal Damage

Urinary Protein Excretion. Urinary protein excretion was determined weekly by nephelometry (Dade Behring III, Mannheim, Germany) by placing the rats in metabolic cages for 24 h (Tecniplast, Bugguggate, Italy).

Focal Glomerulosclerosis. The left kidneys, removed at autopsy, were cut longitudinally, fixed, and processed for paraffin embedding according to standard procedures. Sections of 3 μm were stained with periodic acid Schiff and evaluated microscopically for the incidence of focal glomerulosclerosis (FGS) as described previously (5).

Cardiovascular Parameters

Systolic BP (SBP) was measured weekly in restrained awake animals by means of the tail-cuff method (IITC Inc., Woodland Hills, CA). Infarct sizes were measured in hearts that were removed at autopsy. Mid-sagittal slices of the left ventricle were fixed in Bouin’s solution, embedded in paraffin, and stained with 0.1% Fast Green FCF. Infarct sizes were determined by computerized planimetric measurements as described previously (5).

Statistical Analyses

Data are expressed as mean ± SEM. Concentration-response curves to Ach were expressed as percentage of preconstriction to phenylephrine. The area under each individual curve (AUC) was determined (Sigma Plot, Jandell Scientific, Erkrath, Germany) and expressed in arbitrary units. The AUC was used to represent renal endothelial function of the individual animals. The relationship between the baseline endothelial function and markers of renal damage 16 wk afterward were determined by Kendall nonparametric correlation test using regression analysis (SPSS; SPSS, Inc., Chicago, IL).

Results

Survival

Of 15 rats that underwent MI operation, four died from acute heart failure within 24 h after MI (27%). These rats were excluded from further analysis. The 11 remaining rats were followed during the entire period of 16 wk.

Endothelial Function in the Healthy Nephrectomized Kidney

The renal arteries, isolated at time point 0, from the healthy nephrectomized kidney responded to Ach to a variable extent. The endothelium-dependent relaxation characterized by the Ach AUC averaged 175.6 ± 6.0 arbitrary units, with individual values ranging from 155.0 to 216.9 arbitrary units.

Renal Damage Induced by MI

After UNx and MI, proteinuria gradually increased with time from the mean value of 15 ± 3 mg/24 h at week 0 up to 125 ± 37 mg/24 h at week 16 (P < 0.001; Figure 1). Individual values showed large variation (factor 25.4), ranging from 20 to 507 mg/24 h. The renal damage that was induced by MI in the remaining left kidney was characterized by an increased FGS incidence (17.7 ± 3.4% versus the baseline value of the right healthy kidney 0.8 ± 0.5%; P < 0.001). Furthermore, a marked increase of renal mass/body weight ratio was observed at the end of the study in the left kidney (5.1 ± 0.1 × 10⁻³) as compared with the baseline value of the right healthy kidney (4.1 ± 0.1 × 10⁻³; P < 0.01).

Cardiovascular Parameters

SBP did not increase with time; the values were 133 ± 3 mmHg at week 0 and 131 ± 7 mmHg at week 16 (Figure 1). Histologically assessed MI sizes averaged 25 ± 2%.

Correlation Analysis

Endothelial function that was measured in the healthy kidney that was removed by UNx predicted the renal damage that was inflicted on the remaining kidney by MI. Baseline endothelial function negatively correlated with proteinuria at week 16 (r = -0.62, P = 0.008; Figure 2A), indicating that rats with more pronounced endothelial function developed less proteinuria after UNx and MI. A similar correlation was found between baseline renal endothelial function and FGS at week 16.

Figure 1. Time course of systolic BP (SBP; mmHg) and proteinuria (mg/24 h) in rats that underwent unilateral nephrectomy (UNx; week 0) and myocardial infarction (MI; week 1). Values are given as means ± SEM.
Consecutively, all of the parameters of renal damage were interrelated (proteinuria associated with acute MI. Our data, however, indicate that the renal failure (8,9), the endothelial dysfunction of the systemic arteries that were prepared from the healthy kidney before MI. Consequently, the animals with pronounced endothelial dysfunction occurring after the MI and subsequent heart failure, such as subtotal nephrectomy (4), adriamycin-induced nephrosis (6), or hypertensive-induced renal damage (7). In this study, we found that MI of similar size induces a highly variable damage in the remaining kidney of rats with UNx. The severity of the renal damage was predicted by renal endothelial function that was measured in isolated interlobular arteries that were prepared from the healthy kidney before MI.

It is intriguing that MI of relatively uniform size induces highly variable renal damage that is characterized by both proteinuria and FGS. The considerable variation in proteinuria and renal damage also has been shown for the other models of progressive renal disease despite relative stable and uniform injury, such as subtotal nephrectomy (4), adriamycin-induced nephrosis (6), or hypertensive-induced renal damage (7).

The mechanisms that are responsible for the development of renal damage after MI and UNx are largely unexplored. It is interesting that no proteinuria or histologic damage occurs in animals with MI or UNx alone (5). UNx already represents a state of mild renal damage as the hemodynamic adaptations occur in the remaining glomeruli to compensate for the nephron loss. Several mechanisms by which MI accelerates nephrosis (6), or hypertensive-induced renal damage (7). In this study, we demonstrated that the extent of renal impairment that is induced by MI upon subclinical renal dysfunction is predicted by intrarenal endothelial function before the induction of MI. This observation suggests that measurements of intrarenal endothelial function may be used as a tool to identify individuals who are prone to renal impairment. Furthermore, should renal endothelial function actually determine the sensitivity of the kidney to deleterious events, the modulation of renal endothelial function may provide protection against progressive renal damage. Finally, the progression of the renal damage most likely is (co-)dependent on an intrarenal mechanism, emphasizing further the need for renoprotection in patients with cardiovascular disease. Further exploration of the intrarenal mechanism(s) of the individual susceptibility to renal damage may lead to improved prevention of both cardiovascular mortality and renal function loss.

Acknowledgments

Parts of this study were presented at the 36th Annual Meeting of the American Society of Nephrology, San Diego, CA, November 12 to 17.
2003, and have been published in abstract form (J Am Soc Nephrol 14: 610A, 2003).

We acknowledge the laboratory assistance of J.J. Ducker and the biotechnical assistance of A. Wagenaar.

References