Chapter 3

Endothelial function predicts the development of renal damage after combined nephrectomy and myocardial infarction

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Abstract

Introduction: We have demonstrated that individual renal endothelial dilatory function of the healthy rat predicts susceptibility to subsequent renal damage induced by 5/6 nephrectomy. Additionally, we reported that myocardial infarction (MI) performed upon unilateral nephrectomy (UnX) induced highly variable renal damage. Therefore, we studied whether the variability in renal damage after MI could be explained by the variation in individual renal endothelial function prior to the induction of injury.

Methods: Endothelium-dependent relaxation to acetylcholine was investigated in in vitro in small arteries isolated from the extirpated kidney at UnX. MI was induced one week after UnX by ligation of the left coronary artery. Proteinuria and systolic blood pressure (SBP) were evaluated weekly for 16 weeks thereafter using metabolic cages and the tail-cuff method, respectively. Upon termination of the study, focal glomerulosclerosis (FGS) was evaluated by histology as additional marker of renal damage.

Results: After MI nephrectomized male Wistar rats (n=15) gradually developed variable proteinuria, ranging from 20 to 507 mg/24h at week 16, with an average SBP of 131 ± 7 mmHg. The individual renal endothelial function of the healthy animals predicted the extent of renal damage in terms of proteinuria (r=-0.62, p=0.008) and FGS (r=-0.70, p=0.003).

Conclusions: Individual level of renal endothelial function in the healthy rat is able to predict the severity of renal damage induced by MI. Further exploration of the underlying mechanisms may lead to discovery of preventive renoprotective therapies.
Introduction

The susceptibility of the individuals to develop proteinuria and subsequent renal damage shows large variability. Some individuals develop the renal damage, while others do not. This interindividual variability can not be explained by variation in blood pressure 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 we demonstrated individual differences in the renal endothelial function of the healthy animal to predict severity of renal impairment after subtotal (5/6) nephrectomy\(^4\). Recently, we have 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 hypothesize that the variation in the renal endothelial function among healthy animals predicts their susceptibility to develop proteinuric renal damage after UnX+MI. We employed the rat model of cardiorenal interaction, in which MI is performed following unilateral nephrectomy (UnX). In the healthy kidneys removed at nephrectomy endothelial function of small renal arteries was investigated. Subsequently, this baseline endothelial function was related to markers of renal damage (proteinuria, glomerulosclerosis) measured in the remaining kidney 16 weeks after MI.

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 (250-275 g, n=15, Harlan, Zeist, The Netherlands) were housed under standard conditions. Rats underwent surgical procedures for UnX (followed by \textit{in vitro} measurements of renal endothelial function) and MI. Subsequently, the animals were followed for 16 weeks. Then the left kidney and the heart were removed under anesthesia, weighted and analyzed for the markers of organ damage.

Surgical procedures

\textit{Unilateral nephrectomy (UnX)}

Rats underwent unilateral nephrectomy (UnX) of the right kidney by laparotomy and under anesthesia with isoflurane 3% in \text{N}_2\text{O}/\text{O}_2 (1:2). The removed kidney was weighted, put into cold Krebs solution and one small renal (interlobar) artery per kidney was immediately prepared for measurement of renal endothelial function \textit{in vitro}.
Myocardial infarction (MI)
One week after nephrectomy, rats were intubated, ventilated (Amsterdam Infant Ventilator, Hoek/Loos, Schiedam, The Netherlands), and anesthetized by the administration of isoflurane 3% in N\textsubscript{2}O/O\textsubscript{2} (1:2). Myocardial infarction was induced by ligation of the left ascending coronary artery (LAD) as described previously\textsuperscript{5}. Variation in the infarct sizes was limited by the standard localization of the suture around LAD. Subsequently, the wound was closed and anesthetics replaced by 100% oxygen for a short while until the rat was able to breathe sufficiently on its own.

In vitro measurements of the 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, USA) as described previously\textsuperscript{4} and measured as endothelium-dependent relaxation to cumulative doses of acetylcholine (ACh; \(10^{-8}\) mol/L - \(10^{-4}\) mol/L) in the vessels preconstricted to 45-50% by phenylephrine (\(3\times10^{-7}\) - \(10^{-6}\) 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\textsuperscript{4}.

Markers of the renal damage
Urinary protein excretion
Urinary protein excretion was determined weekly by nephelometry (Dade Behring III, The Netherlands) by placing the rats in metabolic cages for 24h (Tecniplast, Buggugiate, Italy).

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

Cardiovascular parameters
Systolic blood pressure (SBP) was measured weekly in restrained awake animals by means of the tail-cuff method (IITC Inc, USA). Infarct sizes were measured in hearts 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\textsuperscript{5}.

Statistical analysis
Data are expressed as mean ± standard error of the mean (SEM). Concentration-response curves to acetylcholine (ACh) were expressed as percentage of preconstriction to phenylephrine. The Area Under each individual Curve (AUC) was determined (Sigma Plot, Jandell Scientific) 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 weeks afterwards were determined by Kendall non-parametric correlation test using regression analysis (SPSS).

![Graph showing time course of systolic blood pressure (SBP, mmHg) and proteinuria (mg/24h) in rats undergoing unilateral nephrectomy (UnX, week 0) and myocardial infarction (MI, week 1). Values are given as means ± SEM.]

**Figure 1.** The time course of systolic blood pressure (SBP, mmHg) and proteinuria (mg/24h) in rats undergoing unilateral nephrectomy (UnX, week 0) and myocardial infarction (MI, week 1). Values are given as means ± SEM.

**Results**

**Survival**
Out of 15 rats undergoing MI operation, 4 died due to acute heart failure within 24 hours after MI (27%). These rats were excluded from further analysis. Remaining 11 rats were followed during the entire period of 16 weeks.

**Endothelial function in the healthy nephrectomized kidney**
The renal arteries isolated at the time point 0 from the healthy nephrectomized kidney responded to acetylcholine to a variable extent. The endothelium-dependent relaxation characterized by the Area Under the acetylcholine Curve (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/24h at week 0 up to 125 ± 37 mg/24h at week 16 (*Figure 1, p<0.001*). Individual values
showed large variation (factor 25.4), ranging from 20 to 507 mg/24h. The renal damage induced by MI in the remaining left kidney was characterized by an increased FGS incidence (17.7 ± 3.4 % compared with the baseline value of the right healthy kidney 0.8 ± 0.5 %, p<0.001). Further, 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 x 10^{-3}) as compared with the baseline value of the right healthy kidney (4.1 ± 0.1 x 10^{-3}, p<0.01).

**Cardiovascular parameters**

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

![Figure 2. Correlation between the baseline renal endothelial function measured in the healthy nephrectomized kidney and the development of A) proteinuria, B) incidence of focal glomerular sclerosis 16 weeks after the unilateral nephrectomy and myocardial infarction. FGS- focal glomerular sclerosis, AUC- Area Under the acetylcholine Curve in arbitrary units](image)

**Correlation analysis**

The endothelial function measured in the healthy kidney removed by UnX predicted the renal damage inflicted on the remaining kidney by myocardial infarction. The baseline endothelial function negatively correlated with the proteinuria at week 16 (Figure 1A, r=-0.62, p=0.008), indicating that rats with more pronounced endothelial function developed less proteinuria after UnX and MI. A similar correlation was found between the baseline renal endothelial function and focal glomerulosclerosis (FGS) at week 16 (Figure 1B, r=-0.70, p=0.003). Finally, the weight of the kidney measured at autopsy at week 16 was also
predicted by the renal endothelial function at the baseline ($r=-0.59$, $p=0.01$). Consecutively, all the parameters of renal damage were interrelated (proteinuria versus FGS $r=0.96$, $p<0.001$; proteinuria versus kidney weight $r=0.72$, $p=0.01$, FGS versus kidney weight $r=0.60$, $p=0.01$). In contrast, baseline renal endothelial function and the parameters of renal damage did not correlate with either SBP or the infarct sizes (data not shown).

**Discussion**

In the current study, we found that myocardial infarction (MI) of similar size induces a highly variable damage in the remaining kidney of rats with unilateral nephrectomy (UnX). The severity of the renal damage was predicted by the renal endothelial function measured in isolated interlobular arteries prepared from the healthy kidney prior to the MI.

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

The mechanisms responsible for the development of renal damage after MI and UnX are largely unexplored. Interestingly, no proteinuria or histological damage occurs in animals with MI or UnX alone\(^5\). Unilateral nephrectomy (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 mild renal damage may be hypothesized, including neurohumoral activation occurring after the MI and subsequent heart failure\(^8,9\), the endothelial dysfunction of the systemic arteries\(^10,11\) or the generalized inflammatory reaction associated with acute MI. Our data, however, indicate that the renal effects of MI depend, at least partially, on intrarenal factors that are present previous to the injury (MI) triggering renal damage. These factors are reflected by the intrinsic variation in the endothelial dilatory capacity of renal arteries of healthy individuals, as the renal endothelial function predicted the renal outcome after MI. Consequently, the animals with pronounced endothelial dilatory capacity of renal arteries seem to be more protected against proteinuric renal damage induced by an MI.

One of the possible explanations for the relationship between pronounced renal endothelial dilation and protection from injury may be that such vessels have spare capacity to deal with the deleterious hemodynamic alterations associated with neurohumoral activation. The endothelial function of the healthy renal arteries has been already shown to predict proteinuria in the model of subtotal (5/6) nephrectomy\(^4\), in which the glomerular hemodynamic adaptation has also been implicated. It may be suggestive to speculate that similar hemodynamic mechanisms operate in the development of proteinuria in both 5/6Nx and UnX+MI model. Alternatively, the pronounced vasodilatory function may reflect
different endothelial protective properties which deal with other deleterious systemic factors. e.g. the inflammatory response\textsuperscript{12,13}. Finally, even the model of combined UnX and MI represents the complex relation between heart and kidney. Proteinuric disease itself is associated with worsening of cardiovascular prognosis\textsuperscript{14,15} and thus has an impact on the cardiac damage\textsuperscript{16}. Indeed, Dikow et al. has shown increased infarct size in uremic rats\textsuperscript{25}. Although in our study, care was taken to limit the variability of infarct size at the operation, the infarct size was measured at the end of the study and thus under the influence of renal disease. However, the resulting variation was relatively small and the individual infarct sizes did not correlate with either proteinuria or renal endothelial function. Furthermore, another cardiovascular extrarenal parameter, systolic blood pressure, did not change throughout the study and did not correlate with the renal damage. Therefore it is likely that an intrarenal factor was responsible for the variation in renal damage.

In this study we demonstrated, that the extent of renal impairment induced by MI upon subclinical renal dysfunction is predicted by the intrarenal endothelial function prior to the induction of MI. This observation would imply that measurements of intrarenal endothelial function may be used as a tool to identify the individuals prone to the renal impairment. Further, should renal endothelial function actually determine the sensitivity of the kidney to deleterious events, the modulation of the renal endothelial function may provide protection against the progressive renal damage. Finally, the progression of the renal damage is most likely (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.
References


