Chapter 4

Renal endothelial function and blood flow predict the individual susceptibility to adriamycin-induced renal damage

Peter Ochodnický
Robert H. Henning
Hendrik Buikema
Dick de Zeeuw
Richard P.E. van Dokkum

J Am Soc Nephrol 2006; provisionally accepted
Abstract

Background: Susceptibility to renal injury varies among individuals. Previously, we found that individual baseline endothelial function of healthy renal arteries in vitro predicts severity of renal damage after 5/6 nephrectomy. Here we sought to establish this relation in adriamycin-induced nephropathy and questioned whether this predictive value might be detected in vivo as well. We hypothesized that individual differences in endothelial function and renal perfusion predict the severity of adriamycin-induced renal damage.

Methods: Total endothelial relaxation and the contribution of its dilatory mediators prostaglandins, nitric oxide (NO) and endothelium-dependent hyperpolarizing factor (EDHF) were evaluated in small renal arteries isolated from healthy rat kidneys (n=16) using pressurized vessel set-up. In an additional group of healthy spontaneously voiding rats (n=16), baseline glomerular filtration rate- GFR and effective renal plasma flow- ERPF was measured as clearance of iothalamate and para- amino hippuric acid, respectively. Following functional measurements, adriamycin (1.75 mg/kg i.v.) was injected and subsequent renal damage after 6 weeks was related to baseline parameters.

Results: Animals developed highly variable renal damage. Pronounced individual baseline total endothelial and EDHF-mediated relaxation, as well as baseline ERPF was correlated with more severe proteinuria 6 weeks after injection (r= 0.51, p= 0.04; r= 0.68, p= 0.01 and r= 0.66, p= 0.005, respectively). In contrast, baseline NO-mediated dilation was inversely correlated with proteinuria (r= -0.71, p= 0.006).

Conclusion: Individuals with pronounced baseline endothelial dilatory ability measured in vitro and high renal blood flow in vivo are vulnerable to renal damage after adriamycin injection. Therefore interindividual variability in renal hemodynamics might be crucially involved in susceptibility to renal damage.
Introduction

The development and progression of chronic renal damage is largely variable among individuals both in experimental and clinical settings. Environmental systemic factors, such as severity of diabetes or hypertension, cannot fully explain this variation, suggesting some individuals might be intrinsically predisposed to develop renal impairment\(^1\). Several specific animal strains spontaneously develop renal function loss\(^2,3\), indicating that predisposition of an individual to renal damage involves genetically conditioned factors\(^4\). However, variable susceptibility to renal damage could also be observed among individuals within a given animal strain. For instance, after a standardized nephrotoxic challenge, such as 5/6 nephrectomy (5/6Nx), outbred Wistar rats develop renal impairment of highly variable severity\(^5\). Seeking for the factors responsible for this variability we previously observed that \textit{in vitro} measured endothelium-dependent dilatory capacity of small renal arteries in healthy Wistar rats predicts the severity of subsequent renal damage inflicted by 5/6 nephrectomy (5/6Nx)\(^6\). This indicates that intrinsic variability in renal vascular function might be responsible for variable susceptibility to renal injury. However, at present it is unclear whether this finding is specific for hemodynamically-induced renal impairment, such as seen in 5/6Nx, or whether variability in renal vascular function might also be involved in other types of renal injury.

Therefore, in the present study we investigated whether the concept of predictive value of renal endothelial function is valid in a model of nephropathy induced by the nephrotoxic drug adriamycin. In adriamycin nephropathy, a single injection of cytostatic agent adriamycin leads to progressive renal damage with proteinuria, glomerulosclerosis and interstitial damage\(^7,9\). Remarkably, this relatively uniform challenge (standard adriamycin injection), results in largely variable renal damage among individuals\(^10,11\), indicating that some individuals might be more susceptible to adriamycin challenge than others. To elucidate the factors responsible for this variability we measured total endothelium-mediated and specific endothelial mediators (e.g. nitric oxide- NO, endothelium-dependent hyperpolarizing factor- EDHF, prostaglandins- PGs)-dependent relaxation of small intrarenal arteries prior to the administration of adriamycin and related this baseline \textit{in vitro} vascular reactivity to the severity of subsequent renal damage. We also explored whether the concept of renal vascular function predictive value might be confirmed \textit{in vivo}. Therefore, in an additional study we sought to determine whether variability in renal hemodynamic function (GFR, ERPF) measured in conscious healthy rats predicts the nephrotoxic effect of subsequent adriamycin administration. We here report that both renal endothelium-dependent reactivity measured \textit{in vitro} and renal blood flow (ERPF) measured \textit{in vivo} in healthy individuals predict the development of adriamycin-induced nephropathy.
Materials and Methods

The experiments were performed using outbred male Wistar rats (300-350 g, Harlan, Zeist, The Netherlands) housed under standardized conditions in animal facilities of the University of Groningen with free access to food and drinking water. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen.

Study I: Relation between baseline in vitro endothelial function and the severity of adriamycin-induced nephropathy

To investigate the relationship between individual renal endothelial function in vitro and subsequent renal damage in adriamycin-induced nephropathy, rats (n=16) underwent unilateral nephrectomy (UnX) under 3% isoflurane in N\textsubscript{2}O/O\textsubscript{2} anesthesia. Small renal arteries isolated from nephrectomized kidney were employed for in vitro measurements of vascular function as described below. Following UnX, a single i.v. injection of adriamycin in tail vein in a dose of 1.75 mg/kg (Pharmachemie BV, Haarlem, The Netherlands) was administered to induce renal damage. Subsequently, the animals were followed for 6 weeks, during which systolic blood pressure (SBP) was measured weekly in restrained awake animals by means of the tail-cuff method (IITC Inc, Ithaca, USA). Urinary protein excretion was determined weekly by nephelometry (Dade Behring III, Mannheim, Germany) in 24-hour urine samples obtained by putting the animals in metabolic cages. At the end of the study, animals were sacrificed under anesthesia. Remaining kidney and blood samples were harvested for assessment of renal functional and structural damage. Plasma creatinine was measured by means of a colorimetric assay with the Jaffé method without deproteinization (Chema Diagnostica, Jesi, Italy). Focal glomerulosclerosis (FGS) score was determined according to standard procedures in kidneys removed at nephrectomy and autopsy and subjected to fixation and embedding in paraffin. Sections of 3 µm were stained with periodic acid Schiff (PAS) and microscopically evaluated for the incidence of FGS as described previously\textsuperscript{12}.

Measurements of baseline renal endothelial function in vitro

Small renal (interlobar) arteries (250-350 µm) were isolated from the nephrectomized kidney and transferred to an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT, USA) as described previously\textsuperscript{6}. Artery segments were cannulated on glass micropipettes and intraluminal pressure was held constant at 70 mmHg. The vessel chamber was continuously recirculated with warmed (37°C) and oxygenated (5% CO\textsubscript{2} in O\textsubscript{2}) Krebs solution with a pH of 7.4 (120.4 NaCl, 5.9 KCl, 2.5 CaCl\textsubscript{2}, 1.2 MgSO\textsubscript{4}, 25.0 NaHCO\textsubscript{3}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 11.5 mmol glucose). An inverted light microscope
attached to a video camera and video dimension analyzer was used to continuously register lumen diameter.

Following 40 minutes equilibration period, arteries were pre-constricted submaximally with phenylephrine (PE, 3\times10^{-7} - 10^{-6}\text{ mol/l}) and studied for endothelium-dependent and endothelium-independent relaxation by administering cumulative doses of acetylcholine (ACh; 3\times10^{-8}\text{ mol/l} - 3\times10^{-5}\text{ mol/l}) and sodium nitroprusside (SNP, 10^{-9} - 10^{-4}\text{ mol/l}) to the recirculating bath, respectively. ACh-induced relaxation was also studied in the same artery in the presence of either indomethacin (10^{-5}\text{ mol/l}, to inhibit prostaglandins- PGs); indomethacin and N\text{ω}-monomethyl-L-arginine (L-NMMA, 10^{-4}\text{ mol/l}, to additionally inhibit nitric oxide- NO) or indomethacin plus L-NMMA and a combination of charybdotoxin (chtx, 10^{-7}\text{ mol/l}) and apamin (apa, 5\times10^{-7}\text{ mol/l}), applied into the lumen of the artery as well as to the superfusion medium (to additionally inhibit endothelium-derived hyperpolarizing factor- EDHF). ACh and SNP concentration-response curves were successfully obtained in all 16 animals, whereas curves in the presence of all inhibitors were obtained in 13 individuals. Previously, we established that endothelial dilatory function did not differ between renal arteries within the used diameter range isolated from the same kidney and therefore ACh-induced relaxation of one artery can be considered representative.

Study II: Relation between baseline in vivo renal hemodynamic function and severity of adriamycin-induced nephropathy

To investigate whether in vivo determinants of renal hemodynamics in the healthy rat predict subsequent renal damage in adriamycin nephropathy measurements of renal function were performed prior to the injection of adriamycin (1.75 mg/kg) in an additional group of rats (n=16). Following the injection, SBP and urinary protein excretion were measured weekly in the same way as in the first experimental study.

Measurements of baseline renal hemodynamic function in vivo

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by clearance of simultaneously infused iohexol and para-aminhippuric acid (PAH) respectively, in freely moving and spontaneously voiding rats as described previously\textsuperscript{13}. Briefly, the rats instrumented with a jugular and carotid catheter were infused with a bolus of iohexol (9 mg/kg) and PAH (12 mg/kg) followed by continuous intra-arterial infusion of these markers (iothalamate 0.9 mg/h and PAH 4.5 mg/h). Following an initial equilibration period of two hours, clearance period was determined by the urine collection depending on spontaneous voiding of the rat and a blood sample was drawn via jugular catheter after each urine collection. Plasma and urine levels of iohexol and PAH in the samples were determined by HPLC\textsuperscript{14}. ERPF and GFR were calculated as a plasma clearance of PAH and urinary clearance of iohexol respectively, according to the method described by Donker et al.\textsuperscript{15} in man, which was adapted for rats at our laboratory by de Vries et al.\textsuperscript{13}. Mean arterial pressure (MAP) was measured continuously during the
experiment by connecting the carotid catheter to a pressure transducer (Baxter Healthcare, Irvine, USA). Renal vascular resistance was calculated as the ratio of MAP and ERPF.

Table 1. Clinical parameters of animals in both studies measured prior to the administration of adriamycin (baseline) and 6 weeks thereafter and in vivo measurements of renal hemodynamics at baseline in study II.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>320 ± 15</td>
<td>406 ± 22</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135 ± 5</td>
<td>136 ± 11</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>21 ± 6</td>
<td>430 ± 195*</td>
</tr>
<tr>
<td>FGS (%)</td>
<td>0 ± 1</td>
<td>24 ± 10*</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/l)</td>
<td>46 ± 16</td>
<td>69 ± 22*</td>
</tr>
</tbody>
</table>

Renal hemodynamic measurements

| ERPF (ml/min/100g BW)                  | -       | -       | 2.85 ± 0.89 | -       |
| GFR (ml/min/100g BW)                  | -       | -       | 0.53 ± 0.15 | -       |
| MAP (mmHg)                             | -       | -       | 114 ± 15    | -       |
| RVR (mmHg.min/ml)                      | -       | -       | 15 ± 7      | -       |

SBP- systolic blood pressure, FGS- focal glomerulosclerosis, BW- body weight, ERPF- effective renal plasma flow, GFR- glomerular filtration rate, RVR- renal vascular resistance, MAP- mean arterial pressure, ND- not determined; data are presented as mean ± SD; * p<0.05 versus baseline.

Statistical analysis

Data are expressed as mean ± standard deviation (SD), unless stated otherwise. In the analysis of vascular experiments, concentration-response curves to ACh or SNP were expressed in percentage of pre-constriction to PE. The concentrations of drugs causing half-maximal responses were expressed as negative logarithm of the molar concentration (pD<sub>2</sub> values). The Area Under each individual acetylcholine Curve (AUC) was determined (Sigma Plot, SPSS Inc.) and expressed in arbitrary units. The contribution of three endothelial mediators (PGs, NO and EDHF) to endothelial relaxation was calculated as a difference between corresponding AUCs (Figure 1A). Statistical comparisons between
parameters at the baseline and at the end of the study were performed by a Student’s paired t-test. The characteristics of the concentration-response curves were compared by one-way ANOVA or ANOVA for repeated measures when appropriate. Significance was accepted at p<0.05. The relationship between individual endothelial or renal function and renal damage was calculated using Pearson’s parametric or Spearman’s non-parametric correlation test (SPSS), where appropriate.

Figure 1. A) Concentration-response curves to endothelium-dependent vasodilator acetylcholine (ACh) in small renal arteries isolated before the administration of adriamycin. The curves were constructed in absence of any inhibitor (total), in presence of indomethacin (indo, 10^{-5} mol/l), in additional presence of L-NMMA (10^{-4} mol/l) and in additional presence of charbybotoxin (chtx, 10^{-7} mol/l) and apamin (apa, 5x10^{-7} mol/l). Data are given as mean ± SEM. B) Variability in total endothelium-dependent relaxation, prostaglandins (PGs)-, nitric oxide (NO)- and EDHF-mediated relaxation of small renal arteries isolated from healthy rats before the administration of adriamycin. AUC- Area Under Curve expressed in arbitrary units; box whisker plot: central box encloses middle 50% of all the data, horizontal line inside the box represents median and the whiskers encompass 5 to 95 percentiles.

Results

Markers of renal damage in adriamycin nephropathy
Clinical characteristics of the rats used in both studies are presented in Table 1. Six weeks after injection of adriamycin, rats in the first study developed overt nephropathy, characterized by elevated proteinuria, FGS and increased plasma creatinine when compared to values measured in healthy animals before the injection. SBP remained stable over the
entire experimental period. Interestingly, despite the standardized injection of adriamycin, proteinuria varied considerably among individual rats, similarly ranging from 145 to 883 mg/24h in the first and from 124 to 869 mg/24 h in the second experimental group. Both plasma creatinine and FGS score correlated positively with proteinuria (r= 0.56, p< 0.01 and r= 0.63, p= 0.01 respectively), suggesting that proteinuria adequately reflects renal damage in this model.

**Table 2.** Baseline characteristics of the concentration-response curves to endothelium-dependent vasodilator acetylcholine and endothelium-independent vasodilator sodium-nitroprusside (SNP) in small renal arteries isolated from healthy rats prior to the administration of adriamycin. The effect of endothelial vasodilatory pathway inhibitors on the acetylcholine concentration-response curve is also shown.

<table>
<thead>
<tr>
<th></th>
<th>E\text{\textsubscript{max}}</th>
<th>pD\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium-dependent relaxation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine total</td>
<td>80 ± 8</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>82 ± 9</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>+ L-NMMA</td>
<td>64 ± 19\textsuperscript{a,b}</td>
<td>6.0 ± 0.2\textsuperscript{a,b}</td>
</tr>
<tr>
<td>+ indomethacin + L-NMMA</td>
<td>3 ± 2\textsuperscript{a,b,c}</td>
<td>-</td>
</tr>
<tr>
<td>+ chtx+apa</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endothelium-independent relaxation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNP</td>
<td>88 ± 9</td>
<td>6.9 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SD; E\text{\textsubscript{max}}- maximal relaxation to acetylcholine in % of precontraction to phenylephrine, pD\textsubscript{2}- negative logarithm of molar concentration of acetylcholine causing half of maximal response (EC\textsubscript{50}), chtx + apa: charybdotoxin + apamin, \textsuperscript{a} p<0.05 versus control, \textsuperscript{b} p<0.05 versus indomethacin, \textsuperscript{c} p<0.05 versus indomethacin + L-NMMA

**Study I: endothelial function predicts renal damage in adriamycin nephropathy**

*Variability of renal endothelial function in healthy rat*

ACh induced concentration-dependent relaxation of small renal arteries. The average group response and curve characteristics are presented in Figure 1A and Table 2. Relaxation to ACh was highly variable prior to injection of adriamycin in these —at that time— healthy
animals (Figure 1B) and was independent from the level of PE-induced pre-constriction or endothelium-independent relaxation to SNP. Blockade of PGs by indomethacin resulted in variable small changes of the ACh curve in individual rats, however on average not being significantly different (Figure 1A, Table 2). Additional administration of the NO inhibitor consistently decreased endothelium-dependent relaxation (Figure 1A, Table 2), however to a highly variable extent in individual animals (Figure 1B). As a result, the remaining EDHF-mediated relaxation also displayed considerable variability (Figure 1B). This relaxation was completely blocked by the combination of indomethacin, L-NMMA and chtx+apa (Figure 1A, Table 2).

**Figure 2.** A) Correlation between individual total endothelium-dependent relaxation of small renal arteries measured prior to the administration of adriamycin and proteinuria determined 6 weeks after the administration of adriamycin (n=16). B) Correlation between individual relative effective renal plasma flow (ERPF) measured prior to the administration of adriamycin and proteinuria determined 6 weeks after adriamycin injection (n=16). AUC- Area Under Curve expressed in arbitrary units, BW- body weight.

**Correlation analysis**

Correlation analysis was performed to investigate the relation between baseline endothelial dilatory function and the level of renal damage 6 weeks after the administration of adriamycin. ACh-induced relaxation (expressed as AUC) positively correlated with the level of proteinuria (Figure 2A). Endothelium-dependent relaxation also predicted plasma creatinine levels in individual animals (r= 0.68, p< 0.01). Thus, the rats with a pronounced endothelium-dependent dilation at baseline developed more severe renal damage after adriamycin injection. There was no correlation between the individual level of PE pre-
constriction or endothelium-independent relaxation by SNP on one hand and renal damage on the other.

Additionally, we studied the relation between the endothelial mediators of relaxation and proteinuria. PGs-mediated relaxation tended to correlate positively with proteinuria, but this was of marginal statistical significance (Figure 3C). A positive correlation was also found between the individual EDHF-mediated relaxation and proteinuria (Figure 3B). In contrast, individual NO-mediated relaxation was inversely correlated with the level of proteinuria (Figure 3A) as well as FGS (r= -0.69, p= 0.01), suggesting that individuals with a large NO-mediated relaxation are protected from the development of renal damage in adriamycin nephropathy.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Correlation between individual nitric oxide (A), EDHF (B) and prostaglandins (C)-mediated endothelium-dependent relaxation of small renal arteries measured prior to the administration of adriamycin and proteinuria determined 6 weeks after administration of adriamycin (n=13 each). AUC- Area Under Curve expressed in arbitrary units.}
\end{figure}

**Study II: renal blood flow predicts renal damage in adriamycin nephropathy**

Baseline values of renal functional parameters have been included in Table 1. GFR, ERPF and RVR displayed considerable variability in healthy animals prior to adriamycin injection.

Individual ERPF just prior to adriamycin injection markedly correlated with the proteinuria 6 weeks after the induction of the disease (Figure 2B), indicating that individuals with highly perfused kidney at the time of adriamycin administration developed more renal damage. Additionally, a negative correlation was found between individual RVR and proteinuria 6 weeks after adriamycin injection (r= -0.65, p= 0.007). In contrast, no correlation was found between individual baseline GFR and proteinuria at week 6 (r= 0.24, p= NS).
Discussion

In the present study we found that the individual level of renal endothelial function of healthy rats measured *in vitro* predicts their susceptibility to renal damage after injection of adriamycin. Additionally, baseline level of renal blood flow and renal vascular resistance measured *in vivo* were related to adriamycin-induced renal damage as well. These data indicate that state of renal vasculature measured both *in vitro* and *in vivo* might predispose certain healthy individuals to a more severe course of toxic renal damage.

Endothelium-dependent relaxation of intrarenal arteries varies considerably in healthy animals of an outbred rat strain, which allows to test for the predictive value of this parameter in the development of renal damage. In the model of adriamycin nephropathy employed in our study, animals progressively developed highly variable renal damage indicated by increased urinary protein excretion. As shown both in present and previous experiments\(^\text{10}\), proteinuria represents a good indicator of renal damage severity in this model, since it remains relatively stable after 6 weeks and it is correlated with other structural and functional markers of renal damage.

The animals with pronounced baseline acetylcholine-induced relaxation in small renal arteries developed more severe proteinuria after adriamycin injection. This finding might seem surprising since acetylcholine-induced relaxation is considered to be a measure of protective abilities of vascular endothelium\(^\text{16}\) and preserved endothelial relaxation has been shown to be associated with lower rate of future cardiovascular events in high risk populations\(^\text{17,18}\). Furthermore, in contrast to our present data, we previously observed that individuals with large renal endothelial relaxation were protected against renal injury after 5/6Nx\(^6\). It should be noted, however, that the nature of renal injury crucially differs between adriamycin nephropathy and 5/6Nx, involving direct nephrotoxicity in the former and hyperfiltration of remnant nephrons in the latter model\(^5\). A crucial role of the specific etiology of renal injury in adriamycin nephropathy is also suggested by the striking finding of our *in vivo* study. Enhanced baseline level of renal blood flow and reduced renal vascular resistance measured in conscious rats prior to the administration of adriamycin were associated with more severe renal outcome. It has been previously shown that transient clipping of the renal artery for only several minutes prevents adriamycin-induced renal damage\(^10\), indicating that the acute cytotoxic effect of adriamycin in the kidney is responsible for the initial renal injury. Therefore it seems conceivable, that highly perfused kidneys, such as seen in individuals with high ERPF and low RVR, may be exposed to a larger amount of the toxic agent leading to more adverse renal damage. Since endothelium crucially participates in the regulation of renal hemodynamics\(^\text{19,20}\), *in vivo* data might provide a hemodynamic explanation for predictive value of *in vitro* acetylcholine-induced relaxation. However, neural, humoral and local mechanisms other than endothelial participate in the regulation of blood flow *in vivo*. This fact might also contribute to a stronger relation we observed between blood flow and proteinuria than between endothelial
function and proteinuria. Overall, the relation between endothelial function and blood flow on one hand and severity of renal damage on the other hand indicates that the basal state of the renal vasculature, measured both in conscious animals and in isolated preparations, may reflect the susceptibility to renal damage in adriamycin nephropathy. Combined results of our previous (in 5/6Nx)\(^6\) and present (adriamycin nephropathy) data suggest that variability in renal endothelial function is involved in the susceptibility to renal injury under various experimental conditions, however its exact role might be critically dependent on type of renal injury and etiology of progressive renal damage.

To further elucidate contrasting findings of endothelial prediction in diverse experimental models of renal disease we investigated the role of specific mechanisms underlying endothelial relaxation. Acetylcholine-mediated relaxation such as measured in this experiment reflects the sum of functional activity of prostaglandins (PGs), nitric oxide (NO), and EDHF, as evidenced by complete blockade of endothelial response by the combination of respective inhibitors of these pathways indomethacin, L-NMMA and charybdotoxin plus apamin. The view of a protective role of endothelial relaxation is largely based on the concept of beneficial activity of NO\(^16\). Interestingly, when addressing NO-mediated vasodilation specifically, the individuals with large NO relaxation were protected from adriamycin-induced renal damage. A similar relation was also found in rats subjected to 5/6Nx\(^6\), suggesting a protective role of NO in the development of renal injury with various etiologies. Beneficial effects of NO might go far beyond its vasodilatory abilities: NO limits inflammation, proliferation, leucocyte adhesion and other processes involved in the progression of renal damage\(^21\). Several authors propose a crucial role of NO also in the initiation and development of renal injury. Erdely et al. report that mild nitric oxide synthase (NOS) inhibition might convert a rat strain resistant to renal injury after 5/6Nx into a model of rapidly progressing renal disease\(^22\). In humans, several studies have found an association between endothelial NOS polymorphism and end-stage renal disease\(^23,24\). Collectively, these data indicate that variation in NO bioactivity might be crucially involved in interindividual susceptibility to renal injury.

Contribution of additional endothelial mediator, termed EDHF, predicted the development of proteinuria in agreement with total acetylcholine-induced relaxation. EDHF exerts its effects by hyperpolarization of underlying smooth muscle cells, however its identity remains elusive\(^25\). Therefore its role is more difficult to interpret than that of NO. However, one intriguing aspect of EDHF is its putative inverse relationship with NO. Based on this assumption, it was proposed that EDHF might serve as a backup dilatory mechanism under circumstances when NO production is decreased\(^26,27\). Indeed, in present study, individual NO contribution inversely correlated with contribution of EDHF to endothelium-mediated relaxation, thus possibly reflecting the lack of NO-mediated protection. Relaxation mediated by yet another endothelial mechanism, release of prostaglandins (PGs) tended to predict the severity of renal damage. We previously proposed that despite a minimal net effect of cyclooxygenase blockade on acetylcholine-induced relaxation, interindividual variation in the proportion of the relaxing and constrictive PGs might still be detected\(^6\). The
reason for the potential protective role of vasoconstrictive PGs in adriamycin nephropathy remains unclear. One might speculate that predominant release of PGs with constrictive properties in preglomerular vessels may protect glomeruli against a deleterious increase in intraglomerular pressure and hyperfiltration, or could be involved in the regulation of renal blood flow during adriamycin injection. Overall, these data suggest that measurement of endothelium-dependent relaxation attributed to specific mediators, such as NO, EDHF and PGs, might provide additional important information on the interindividual susceptibility to renal damage.

**Perspectives**

If consistently confirmed, the predictive value of renal vascular function might have broad potential clinical implications. Measurements of baseline endothelial or hemodynamic function and organ blood flow might identify individuals with increased risk for future adverse renal outcomes. More important, one may speculate on interventional strategies to prevent end-organ damage, for instance by specifically targeting NO, EDHF and/or cyclooxygenase pathways. Additionally, findings in a model of adriamycin nephropathy might have potential implications for adriamycin-induced long-term organ toxicity associated with chemotherapy in humans. In addition to nephrotoxicity, severe cardiomyopathy often manifests after adriamycin treatment in humans. Whether interindividual differences in endothelial function and/or organ perfusion predict the extent of damage also in this condition, needs to be investigated further. If so, then lowering blood flow to a specific organ at the time of drug administration may provide a protection from unwanted toxicity.

**Conclusion**

In this study, we showed that both baseline endothelial function of isolated renal vessels and renal blood flow measured *in vivo* in conscious animals predict the severity of renal damage imposed by subsequent administration of a nephrotoxic drug. Together with previous findings in other experimental models of renal damage, these data suggest that interindividual variability in baseline renal hemodynamics might be responsible for susceptibility to renal impairment. The predictive value of total renal endothelial function seems to be critically dependent on the etiology of renal injury, whereas nitric oxide-dependent relaxation provides the consistent information in different experimental models. Further investigation into the nature of renal vascular variability may help us to reveal the mechanisms involved in the development of progressive renal disease.
References


