Nephrol Dial Transplant (2008) 23: 1854–1860
doi: 10.1093/ndt/gfm911
Advance Access publication 24 January 2008

Original Article

NDT
Nephrology Dialysis Transplantation

Vascular dysfunction in adriamycin nephrosis: different effects of adriamycin exposure and nephrosis

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Abstract

Background. Nephrosis-induced endothelial dysfunction is assumed to play a main role in cardiovascular morbidity. Adriamycin-induced proteinuria is a well-established rat model for nephrotic syndrome. However, induction of nephrosis by intravenous adriamycin administration might exert direct adriamycin cardiovasculotoxicity that could obscure or modify nephrosis-induced vascular dysfunction. The present study, therefore, investigated *in vitro* vascular function in the isolated thoracic aorta and isolated perfused hearts of rats with adriamycin nephrosis, as compared to non-nephrotic adriamycin exposed rats.

Methods. Adult rats received a single slow intravenous injection of either adriamycin (1.5 mg/kg, adriamycin nephrotic rats) or saline (healthy controls). In a third group of rats, the cardiovascular system, but not the kidneys, were exposed to adriamycin by transient clipping of renal arteries during adriamycin injection (adriamycin control rats).

Results. Exposure of the kidneys to adriamycin induced severe proteinuria with corresponding systemic nephrosis, as apparent from hypercholesterolaemia. Adriamycin exposure of the vascular bed led to marked blunting of the aortic response to the endothelium-dependent vasodilator, acetylcholine (ACh), both in non-nephrotic and nephrotic rats. The nephrotic state reduced the bradykinin-induced increase in coronary flow and enhanced the aortic constrictor response to angiotensin II associated with reduced basal aortic NO-activity, as shown by the comparison between adriamycin nephrotic rats and healthy and adriamycin controls.

Conclusions. Vascular adriamycin exposure and nephrosis affect vascular function in a distinct and qualitatively different fashion in adriamycin-induced nephrotic syndrome. The differential effects of nephrosis and vascular adriamycin exposure have to be accounted for in the interpretation of vascular studies in adriamycin nephrosis.

Keywords: adriamycin; nephrosis; vascular dysfunction

Introduction

Anthracyclines are widely used for the therapy of a variety of malignant diseases, but their therapeutic dose is limited by severe side effects such as myelosuppression and cardiotoxicity. Anthracyclines such as daunorubicin and adriamycin (doxorubicin) are also established tools to induce experimental nephrosis, the onset and severity of which depend on the total drug dose used and dosage schedule employed [1,2]. In the rat, a single intravenous injection of a relatively low-dose of adriamycin (1.5 mg/kg) causes glomerular cell injury leading to a gradual increase in proteinuria and subsequent/concomitant changes in plasma cholesterol until 4–8 weeks, after which it remains relatively stable. After several weeks, glomerular sclerotic lesions may be detected. And, as this occurs in the absence of hypertension and without marked early renal function loss [3], the rat adriamycin nephrosis model provides a relatively 'pure' experimental model to study proteinuria-induced glomerulosclerosis and target organ damage in the nephrotic syndrome. Employing the model as such, we previously found that, rather than the severity of proteinuria, the state of systemic nephrosis with dyslipidaemia is a main determinant of the severity of renal damage [4].

Such findings emphasize the importance of systemic nephrosis in the pathophysiology of target organ damage in this model. It could therefore also provide a model to study nephrosis-related vascular dysfunction. Hence, this seems to be of significant importance because nephrosis-induced vascular, and particularly endothelial, dysfunction is believed to play a main role in the cardiovascular morbidity and mortality in the nephrotic syndrome. The rat anthracyclin-induced nephrosis model has indeed been used to study nephrosis-related endothelial dysfunction. Ito *et al*., for example, reported significant blunting of acetylcholine (ACh)-induced endothelium-dependent relaxation in aortas isolated from rats with nephrosis that was induced

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by daunorubicin, and suggested a possible role for hypercholesterolaemia herein [5]. Furthermore, an association between lipid/lipoprotein abnormalities and endothelial dysfunction in experimental nephrosis has been shown previously [6]. One possible drawback, however, could be that induction of nephrosis by intravenous anthracyclin administration might exert a direct vasculotoxicity that could obscure or modify nephrosis-induced vascular dysfunction leading to misinterpretations of the findings. Therefore, the aim of the present study was to investigate whether, in the model of adriamycin-induced nephrotic syndrome, direct vasculotoxic effects of adriamycin nephrosis are present that could obscure or modify nephrosis-induced vascular dysfunction. To this end, we investigated *in vitro* vascular function in the isolated thoracic aorta and isolated perfused hearts of rats with nephrotic syndrome induced by a single intravenous injection of adriamycin. Rats in whom the renal arteries were temporarily clipped to prevent the kidneys from exposure to adriamycin during injection (i.e. non-nephrotic adriamycin-exposed rats) and normal rats without adriamycin exposure served as controls.

Methods

Animals

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.

Nephrotic syndrome was induced by a single adriamycin injection in the penis vein (Pharmachemie BV, Haarlem, Holland) in male Wistar rats (Harlan, Zeist, The Netherlands) as described previously [4]. This procedure results in a moderate to severe proteinuria that levels off at 6 weeks after injection and remains more or less stable up to 12 weeks after injection [3,4,7]. The rats received a single slow injection of adriamycin ($n = 12$, 1.5 mg/kg; adriamycin nephrotic rats: AN) or vehicle (saline, $n = 12$, healthy controls: CON) in the penis vein. In 12 other adriamycin rats, serving as controls for the vascular effects of adriamycin in the absence of a nephrotic state, both renal arteries were clipped for 12 min during the adriamycin injection after having been exposed by a midline laparotomy (adriamycin controls: AC). Previous studies have shown that this clipping procedure does not lead to renal damage or proteinuria [4,8,9]. A midline laparotomy was also performed in AN and CON rats without renal artery clipping (i.e. sham operation).

During the study, the animals were housed in group cages in a temperature-controlled room with a fixed light-dark cycle. They received a low-sodium diet (0.05% NaCl, 20% protein, Hope Farms Inc., Woerden, The Netherlands) and daily fresh tap water ad libitum. During the 12-week study, 24-h urine was collected from rats kept in metabolic cages with free access to food and water. Body weight and blood pressure were measured twice a week. At the end of the 12-week period, rats were anaesthetized and 500 IU of heparin was given intravenously (tail vein) before plasma sampling. The heart and aorta were rapidly excised for functional measurements *in vitro*.

Blood pressure measurements

Animals were trained prior to the experiments to get accustomed to the measurement procedure. To rule out interindividual differences and environmental influences, all measurements were performed by the same observer in one single room with no other animals around. Systolic blood pressure was measured in conscious rats with an automated multichannel system (Apollo 179, IITC, Life Science, Woodland Hills, CA, USA). This system used tail cuffs and photo-electric sensors to detect tail pulse. To this purpose, the rats are placed in the test chamber in restrainers appropriate for their size and body weight. During each blood pressure measurement session five measurements were recorded for each animal. Blood pressure was taken as the mean of the last three recordings.

Biochemistry

Urinary protein was determined by the Biuret method (Bioquant, Merck, Darmstadt, Germany). Plasma cholesterol concentration was determined by an enzymatic colourimetric test (CHOD-PAP, Boehringer Mannheim, Germany).

Flow measurements in the isolated heart

After removal, the heart was arrested in icy cold 0.9% NaCl. Retrograde perfusion of the aorta, essentially by the Langendorff method, was achieved immediately using a modified Tyrode solution equilibrated with 95% O_2 and 5% CO_2 [10]. This buffer was filtered through a 1.2-μm pore-size filter before reaching the heart. Perfusion was maintained at 60 mmHg and temperature (measured at the aortic cannula tip) was kept between 38.0 and 38.5°C. The hearts beat spontaneously throughout the experiments. Coronary flow (volume of perfusion fluid per unit of time) was measured by a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic perfusion pump. After an equilibration period of 10 min, baseline measurements were made and subsequently concentration-response relations were determined with increasing concentrations of bradykinin (3, 10 and 30 nmol/l) [10]. Each concentration was added to the perfusion fluid and maximal changes in coronary flow were measured within 2–3 min. Between two consecutive exposures to bradykinin a 5-min washout and stabilization period was introduced. Bradykinin stimulates the endothelium to produce/release vasodilator substances (including NO) that act on underlying coronary smooth muscle cells to cause dilation and increase in flow. Hence, endothelium-dependent bradykinin-induced coronary flow responses were used as functional indices for endothelial function. After addition of the highest concentration of bradykinin, a 10-min washout and stabilization period was allowed before 10 mmol/l sodium nitrite (SN) was added [10]. SN is an exogenous NO-donor that directly acts on coronary smooth muscle cells to cause dilation. Hence, endothelium-independent SN-induced maximal increase in coronary flow was used to control for coronary smooth muscle cell relaxation to NO *per se*.

Contraction measurements in the isolated aorta

Immediately after removal, the aorta was placed in a Krebs bicarbonate solution equilibrated with 95% O_2 and 5%

CO_2 as described previously [11]. After the removal of connective tissue, two parallel rings of 2 mm length were cut with a sharp razor blade, with care not to disrupt the integrity of the endothelium. The rings were mounted in 15 ml organ baths filled with the Krebs solution at 37.5°C and connected to a displacement transducer to determine isotonic changes [10]. The rings were subjected to 14 mN and allowed to stabilize for 60 min during which regular washing was performed. They were primed by evoking a contraction with 1 μmol/l phenylephrine (PE) followed by repeated washings and repeated stabilization before entering the protocol.

In the first series of measurements, both rings were pre-contracted with 1 μmol/l PE followed by cumulative administration of increasing concentrations of ACh (10 nmol/l to 0.1 mmol/l). ACh stimulates the endothelium to produce/release vasodilator substances (including NO) that act on underlying vascular smooth muscle cells to cause dilation. Hence, endothelium-dependent ACh-induced dilatory responses were used as functional indices for endothelial function.

In the second series of measurements—after washing and stabilization—again both rings were pre-contracted with 1 μmol/l PE but now followed by cumulative administration of increasing concentrations of SN (10 μmol/l to 10 mmol/l). SN is an exogenous NO-donor that directly acts on vascular smooth muscle cells to cause dilation. Hence, endothelium-independent SN-induced dilatory responses were used to control for vascular smooth muscle cell relaxation to NO *per se*.

After washing and renewed stabilization, one of the rings was incubated with vehicle (saline) and the parallel ring with 100 μmol/l N^{ω}-monomethyl-L-arginine (L-NMMA) for 30 min. With these drugs still present, both rings were then stimulated with increasing concentrations of angiotensin II (AngII; 0.1 nmol/l to 1 μmol/l) in the third series of measurements [10,11]. L-NMMA prevents the NO-synthase from producing NO, thereby abolishing the inhibitory effect of basal NO-release on contractile responses to AngII. Comparisons with AngII-induced contractions in the absence of L-NMMA were used as indices for basal NO-activity.

Data analysis

Duplo relaxatory responses to ACh and SN were averaged per rat and calculated as the percent change of PE-induced pre-contraction. For assessment of the effect of L-NMMA on the constrictor responses to AngII, area size for the dose–response curves in the presence or absence of L-NMMA was determined. To this purpose, first, curves were normalized to allow for group comparisons; the maximal response to AngII (μmol) in the presence of L-NMMA was set at 100% and the corresponding responses to AngII in the absence of L-NMMA were calculated accordingly. Subsequently, the area between the normalized curves to AngII in the presence and absence of L-NMMA was determined, i.e. the Δarea [10]. Baseline coronary flows (ml/min) as well as bradykinin and SN-induced increases in coronary flow were corrected for heart weight (ml/min/g).

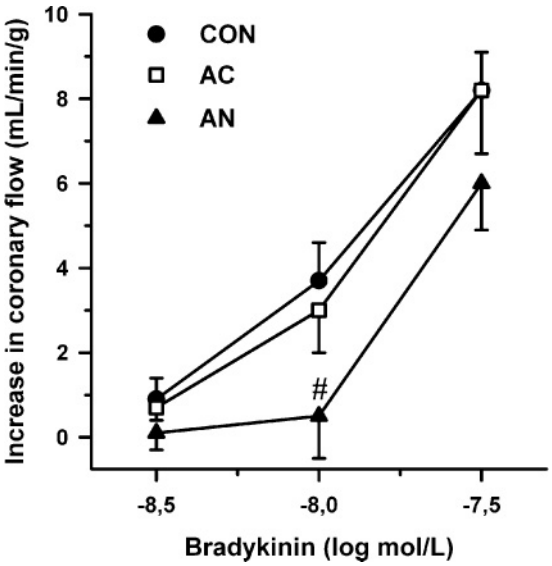

Fig. 1. Increase in coronary flow after stimulation with the endothelium-dependent vasodilator bradykinin in the isolated Langendorff-perfused hearts of control (CON), adriamycin control (AC) and adriamycin nephrotic (AN) rats. Data are presented as means ± SEM. # indicates $P < 0.05$ versus CON.

Data are presented as means ± SEM unless stated otherwise. One-way ANOVA (and repeated measures ANOVA in the case of dose-response profiles) was used for primary evaluation of group means in combination with *post hoc* (Bonferroni-corrected) *t*-statistics. Significance was assumed at *P*-values <0.05 (two-tailed).

Results

General characteristics

In rats with adriamycin exposure of the kidneys, a gradually increasing proteinuria was observed that levelled off at 5 to 6 weeks after injection and remained more or less stable until termination at Week 12. A slightly elevated urinary protein excretion in the non-nephrotic range was observed in the adriamycin controls. Data at Week 12 are given in Table 1. It shows that blood pressure was normal in all groups. The proteinuria in the adriamycin nephrotic rats was accompanied by corresponding elevations in serum cholesterol, our index of systemic nephrosis. Cholesterol was normal in healthy controls and in adriamycin controls. In the adriamycin groups body weight was slightly below that in healthy controls.

Flow measurements in the isolated heart

Baseline coronary flow (ml/min/g) was not significantly different among the groups (15.4 ± 1.2 for CON, 15.6 ± 0.5 for AN and 14.8 ± 1.5 for AC; one-way P = NS). Perfusion of the heart with the endothelium-dependent vasodilator bradykinin caused concentration-dependent increases in coronary flow. This increase did not significantly differ between healthy controls and adriamycin controls, but was significantly decreased in adriamycin nephrosis rats (Figure 1). On the other hand, a maximal increase in

Table 1. Clinical parameters at the end of the study

	Healthy controls ($n = 12$)	Adriamycin nephrosis ($n = 12$)	Adriamycin controls ($n = 12$)
Body weight (g)	522 ± 8	$481 \pm 10^{\#}$	$482 \pm 14^{\#}$
Systolic blood pressure (mmHg)	140 ± 3	142 ± 7	139 ± 3
Proteinuria (mg/day)	30 ± 7	$511 \pm 63^{\#}$	$89 \pm 22^{\#*}$
Plasma cholesterol (mM/L)	2.2 ± 0.1	$5.9 \pm 0.7^{\#}$	$2.3 \pm 0.2^{*}$

Data are given as means ± SEM. $^{\#}P < 0.01$ adriamycin nephrotic rats or adriamycin controls versus healthy controls; $^{*}P < 0.01$ adriamycin controls versus adriamycin nephrosis.

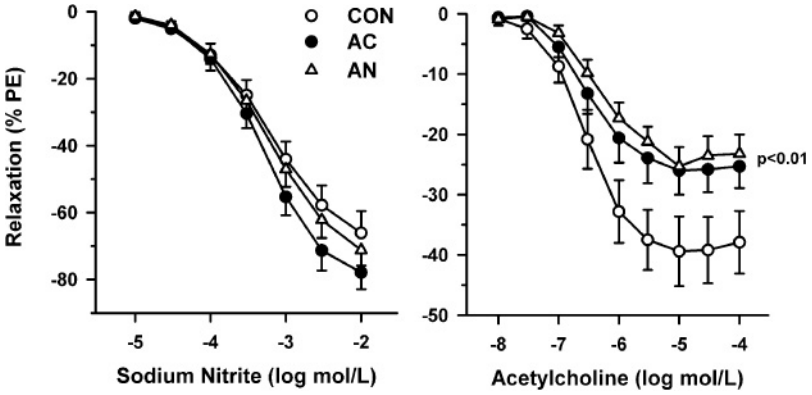

Fig. 2. Relaxation to endothelium-independent vasodilator sodium nitrite (left panel) and endothelium-dependent vasodilator acetylcholine (right panel), as a percent of pre-contraction to phenylephrine (PE), in control rats (CON), adriamycin controls (AC) and adriamycin nephrosis (AN). Data are presented as means ± SEM.

coronary flow induced by the endothelium-independent vasodilator SN did not significantly differ among the groups (7.8 ± 0.6 for CON, 6.5 ± 1.3 for AN and 7.2 ± 0.9 for AC; one-way $P =$ NS), indicating that coronary flow reserve was unaffected.

Vascular measurements in the isolated aorta

The contractile responses to 1 μmol/l phenylephrine, used to elicit a state of pre-contraction for the vasodilator experiments, were not significantly different for the three groups, being 284 ± 27 mN in adriamycin nephrosis, 289 ± 15 mN in adriamycin controls and 285 ± 20 mN in healthy controls. Pre-contraction was similar for all series of measurements with vasodilators.

The dilator responses to ACh and SN are shown in Figure 2. It shows that in adriamycin nephrosis and adriamycin controls, the response to ACh (right panel) was significantly below control (both $P < 0.001$). The responses to SN (left panel) were slightly elevated in both groups as compared to control, indicating that the impaired ACh responses in adriamycin nephrotic rats and adriamycin controls cannot be attributed to impaired relaxatory capacity. To account for the differences in SN response in the interpretation of the ACh responses, the ratio of these responses was calculated, as shown in Figure 3. It shows that the ACh/SN response ratio was significantly below the values in healthy controls for adriamycin nephrotic rats as well as adriamycin controls, indicating impairment of endothelium-dependent vasorelaxation in these two groups.

Vasoconstrictor responses to AngII are given in Figures 4 and 5. The maximal responses to AngII (Figure 5, left

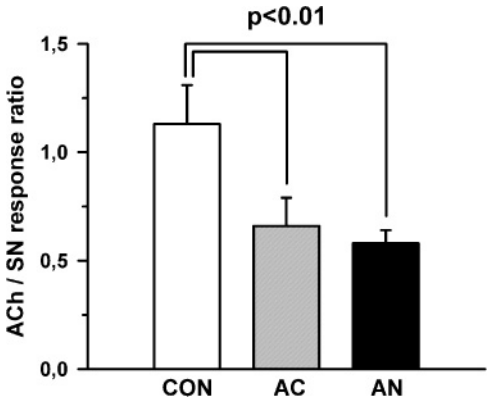

Fig. 3. Ratio of the responses to endothelium-dependent vasodilator acetylcholine (ACh) to endothelium-independent vasodilator sodium nitrite (SN) in control rats (CON), adriamycin controls (AC) and adriamycin nephrosis (AN). The ratio was calculated as the ratio of the areas under the curve for the responses to ACh and SN, respectively. Data are given as means ± SEM.

panel) were significantly greater in the nephrotic rats—but not in the adriamycin controls—as compared to the healthy control rats. For that matter, there was a positive linear correlation between the maximal response to AngII and log proteinuria ($r = 0.548$, $P = 0.001$). After pre-incubation with L-NMMA (Figure 5, right panel), the vasocontrictor responses to AngII were similar for the three groups. The effect of pre-incubation with L-NMMA on the dose response to AngII in the different groups is given in Figure 4. It shows that pre-incubation with L-NMMA did not significantly alter the response to AngII in nephrotic rats, but

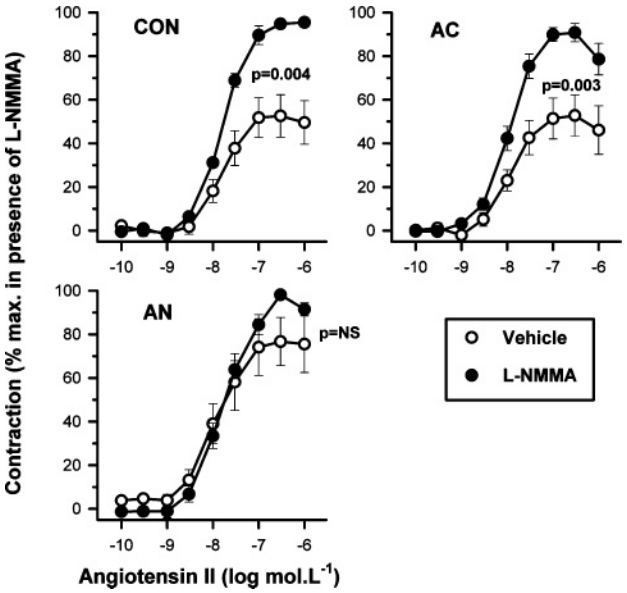

Fig. 4. Dose responses to AngII in control rats (CON), adriamycin controls (AC) and adriamycin nephrotic rats (AN), without (vehicle, open circles) and with NO inhibition (LNMMA, closed circles) by pre-incubation with L-NMMA. The maximum contraction during pre-incubation with L-NMMA was taken as a reference value (i.e. set at 100%) and the responses of corresponding parallel rings in the absence of L-NMMA were calculated accordingly. Data are given as means ± SEM.

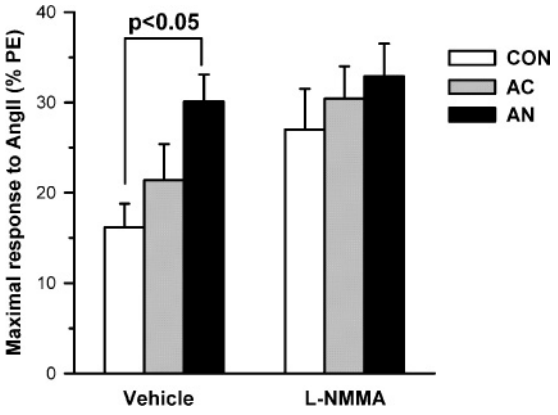

Fig. 5. Maximal vasoconstrictor responses to AngII (as a percent of the vasoconstrictor response to phenylephrine) for control rats (CON), adriamycin controls (AC) and adriamycin nephrotic rats (AN), in the absence (vehicle, left panel) and in the presence (L-NMMA, right panel) of L-NMMA. Data are given as means ± SEM.

significantly enhanced the responses to AngII (at concentrations ≥10 nmol/l) in the other groups. Thus, preincubation with L-NMMA annihilated the difference in the contractile response to AngII between the nephrotic rats and the other groups, and the correlation between the maximal response to AngII in the presence of L-NMMA and log proteinuria no longer persisted ($r = 0.252$, $P = \text{NS}$).

Discussion

As adriamycin is known to have cardiovascular effects, the aim of the present study was to investigate whether, in the model of adriamycin-induced nephrotic syndrome, direct vasculotoxic effects of adriamycin exposure are present that could obscure or modify possible effects of the nephrotic state. Our data demonstrate differential effects of adriamycin exposure and of the nephrotic state, respectively, on the vascular bed. Accordingly, when using the adriamycin model for analysis of the cardiovascular effects of nephrosis, the effects of adriamycin exposure will have to be accounted for. Adriamycin exposure of the vascular bed as such led to the marked blunting of the aortic response to the endothelium-dependent vasodilator ACh, both in non-nephrotic and nephrotic rats. The nephrotic state as such reduced the bradykinin-induced endothelium-dependent increase in coronary flow and enhanced the aortic constrictor response to AngII associated with a reduced basal NO release, as apparent from the comparison of adriamycin nephrotic rats versus healthy and adriamycin controls.

In the present study, impaired endothelium-dependent relaxation to ACh in aortas of adriamycin controls as compared to healthy control rats was found together with intact endothelium-independent relaxation to exogenous NO. Since the relaxant effect of ACh in rat aorta is largely mediated by NO released from the endothelium [12], such findings are suggestive for an impairment of stimulated endothelial NO formation, release or activity. Alternatively, altered endothelial cell receptor-coupling mechanisms might be involved in the blunted ACh response, and we also cannot formally exclude impairment in other endothelial vasodilator pathways (i.e. EDHF, prostaglandins) or a direct contractile effect on the vascular smooth muscle level of the agonist employed. Nevertheless, as the impairment was present in adriamycin controls in the absence of overt proteinuria it seems attributable to *in vivo* vascular adriamycin exposure as such. The preserved responses to the (cGMP-mediated) endothelium-independent vasodilator, SN, exclude general defects in responsiveness to exogenous NO and in relaxatory capacity at the vascular smooth muscle level in these animals.

The action mechanisms of adriamycin in relation to NO and endothelial dysfunction, as brought up in the literature, include the enzymatic one-electron reduction of adriamycin. This forms the adriamycin semiquinone radical, which rapidly reacts with oxygen to form superoxide and (regenerated) adriamycin. It has also been reported that adriamycin may bind to, and is reduced by, the reductase domain of endothelial nitric oxide synthase (eNOS), thereby diverting electron flow away from the oxygenase domain of the enzyme. As a consequence, superoxide formation is dramatically increased and NO formation decreased [13]. Findings from recent *in vitro* studies suggest, however, that exposure to adriamycin does not necessarily lead to acute impairment of endothelial relaxant function, but rather to its impairment over time. Den Hartog *et al*. [14] in an *in vitro* study found no inhibition by doxorubicin (adriamycin) of eNOS in the isolated rat aorta. Murata *et al.* [15], who assessed the effect of 'prolonged' doxorubicin exposure (i.e. several days) on vascular endothelium in an organ culture study, reported attenuation of endothelium-dependent relaxation no sooner than at the fifth day of the treatment. Our present results may extend these findings, suggesting that

in vivo exposure to adriamycin may blunt aortic ACh-induced, NO-mediated relaxation over time.

In contrast, basal vascular NO may be more resistant to adriamycin as suggested by our present findings with AngII. The contractile response to AngII, like that of other vasoconstrictor hormones, is counteracted by basally released endothelial NO [16]. Since AngII-induced contraction was comparable in aortas of adriamycin controls and healthy control rats and, similarly, enhanced in both groups after inhibition of eNOS, this suggests that *in vivo* adriamycin exposure did not affect aortic basal NO activity. Together with the above it also implies that basal and stimulated NO release/activity may become differentially affected by adriamycin. Our findings with isolated perfused hearts additionally suggest that the vascular system may not become uniformly attenuated by adriamycin. In contrast to the aorta, endothelium-dependent agonist-induced relaxations and increases in coronary flow in isolated hearts were not attenuated in adriamycin control rats. Although comparison between both for interpretation of the results is somewhat hindered here because of the difference in the agonist employed, i.e. ACh in the aorta versus bradykinin in the heart, our findings do suggest that different vessel beds and/or artery types may be differentially susceptible for exposure to adriamycin.

Adriamycin injection without temporary clipping of the renal arteries resulted in overt nephrotic sydrome. Adriamycin nephrotic animals were characterized by proteinuria and hypercholesterolaemia, both of which have been shown to be a risk marker for endothelial dysfunction and to be associated with its development [17]. In keeping with a previous study, employing an anthracyclin-induced rat model of nephrosis [5], we found the relaxant aortic response to ACh to be blunted in adriamycin nephrotic rats. However, this blunting was similar to that observed in adriamcyin control rats in the present study, thus, without any further impairment exerted by the nephrotic state as it appears. While the above-mentioned study suggests nephrosis to attenuate endothelium-dependent relaxation, but in which study a non-proteinuric adriamycin control group is lacking, our present findings with the same vascular bed, also using an anthracyclin rat model, would not allow the same conclusion to be drawn. The possibility that the impaired aortic relaxant response was due to slow-onset adriamycin vasculotoxicity, rather than the nephrotic syndrome, indicates an important drawback of the anthracyclin model for studies of the development of endothelial dysfunction in rats with experimental nephrosis.

It should be emphasized, however, that a potential deleterious effect of the nephrotic state on endothelial function (as is generally believed) still is not to be excluded in the above. Hence, the blunting in ACh-induced relaxation by *in vivo* adriamcyin exposure (e.g. as found in adriamycin controls) might have masked a potential blunting of endothelial function by nephrosis factors. In fact, our present findings also provide evidence that would support such a role of nephrosis. A bradykinin-induced endothelium-dependent increase in coronary flow in the isolated perfused heart was significantly decreased in adriamycin nephrotic rats as compared to adriamycin controls, and also the aortic constrictor response to AngII was significantly enhanced in nephrotic rats. Furthermore, the latter response was without a further significant enhancement by L-NMMA, which suggests that nephrosis was associated with reduced basal NO activity to oppose aortic vasoconstriction. AngII-induced contractility might also have been modulated by alterations in the angiotensin type II receptor (AT2R) density and function, as has indeed been reported under other pathophysiologic conditions as a compensatory mechanism [18]. Since we did not further study the effect of AT2R blockade in this model, its involvement in the present study cannot be excluded. Nevertheless, our findings are in keeping with previous reports of enhanced contractility in this model to other vasoconstrictors [19], suggesting that nephrosis may be associated with a decrease in basal release or activity of endothelium-derived relaxing factors.

In conclusion, in adriamycin-induced nephrotic syndrome, vascular adriamycin exposure and nephrosis affect vascular function in a distinctly and qualitatively different fashion. Adriamycin exposure reduces endothelium-dependent vasodilation, most likely due to impaired stimulated NO release without further impairment by nephrosis. Nephrosis, on the other hand, is associated with a reduced basal NO release—without an additional effect of vascular adriamycin exposure—leading to impairment of the normal vascular modulation of vasoconstrictor stimuli. The latter is an important vascular defense mechanism [20] and its impairment might well be involved in the high cardiovascular morbidity in proteinuric patients. The differential effects of nephrosis and vascular adriamycin exposure as well as the heterogeneity of effects across the vascular bed have to be accounted for in the interpretation of vascular studies in adriamycin nephrosis.

Acknowledgements. We gratefully acknowledge the technical support of A. van Buiten. The study was supported by a grant (C95.1495) of the Dutch Kidney Foundation (Nier Stichting Nederland).

Conflict of interest statement. None declared.

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Received for publication: 3.7.07
Accepted in revised form: 3.12.07