Human recombinant erythropoietin augments serum asymmetric dimethylarginine concentrations but does not compromise nitric oxide generation in mice

Anjali Desai, Ying Zhao and Jeffrey S. Warren

Department of Pathology, University of Michigan Medical School, 1301 Catherine Street, Ann Arbor, MI 48109-0602, USA

Abstract

Background. Patients with advanced chronic renal disease (CRD) suffer from excessive morbidity and mortality due to complications of accelerated atherosclerosis. Recombinant human erythropoietin (EPO), which is routinely used to treat the anaemia present in ~90% of dialysis-dependent patients with end-stage renal disease, may induce vascular dysfunction by reducing nitric oxide (NO) availability. Pathophysiologic concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), are found in patients with CRD and correlate with vascular disease and cardiovascular mortality. The aim of the current study was to investigate the effect of EPO on ADMA concentrations and NO generation in vitro and in vivo. Furthermore, we wanted to study the effect of EPO on the expression of the enzymes that regulate ADMA metabolism and NO generation.

Methods. Human umbilical vein endothelial cells (HUVECs) were exposed to therapeutic concentrations of EPO. The expression and metabolic activity of dimethylarginine dimethylaminohydrolase II (DDAH II), the enzyme that degrades ADMA, was evaluated. Following subcutaneous administration of EPO to Balb/c mice for 10 weeks, serum ADMA concentrations were determined. Systolic blood pressure was measured noninvasively. Urinary nitrite and nitrate (NOx) concentrations were assessed by Griess assay. Protein expression of DDAH and NOS in livers and kidneys was measured by western blotting.

Results. EPO suppressed ADMA elaboration by HUVECs. Systolic blood pressure and serum concentrations of ADMA were significantly elevated in EPO-treated mice. The protein expression of DDAH I in the kidney and liver was upregulated while hepatic expression of DDAH II was decreased and renal DDAH II expression remained unchanged by EPO administration. However, EPO augmented urinary NOx concentrations as well as the expression of NOS I and NOS 2 in the kidney.

Conclusion. In spite of elevating serum ADMA concentrations, EPO does not appear to compromise overall NO generation in Balb/c mice.

Keywords: dimethylarginines; dimethylarginine dimethylaminohydrolase; endothelial cells; erythropoietin; nitric oxide

Introduction

Cardiovascular mortality is 30 times greater in patients with chronic kidney disease (CKD) than in the general population [1]. Reduced nitric oxide (NO) bioavailability, observed in patients with CKD, is linked to accelerated vascular disease [2]. Recently it has been proposed that the endothelial dysfunction observed in patients with CKD is a consequence of increased plasma concentrations of asymmetric dimethylarginine (ADMA) [3]. ADMA is the predominant endogenous inhibitor of NOS and is now widely recognized not only as a uraemic toxin but as a global marker of cardiovascular risk [4]. Pathophysiologic levels of ADMA are found in patients with CKD and correlate with vascular disease and cardiovascular mortality. Thum et al. reported that in patients with stable angina, plasma ADMA concentrations were related to the severity of coronary artery disease and correlated inversely with the number of circulating CD34+/CD133+ progenitor cells and endothelial colony forming units [5]. Furthermore, ADMA inhibited the mobilization, differentiation and function of cultured endothelial progenitor cells. Recently Zoccali et al. reported that ADMA is a powerful independent predictor of cardiovascular mortality in haemodialysis patients [6]. In fact, ADMA levels were stronger predictors of cardiovascular outcome than some traditional risk factors such as systolic pressure or total and LDL cholesterol.

Recombinant human erythropoietin (EPO), which is widely used to treat anaemia in kidney disease and other forms of nonrenal anaemia, has recently received a heightened level of attention in the media and in the medical community [7]. A clinical trial involving more than 1400
patients with CKD revealed that administration of higher doses of EPO (targeted to achieve a haemoglobin level of 13.5 g/dl) were associated with increased risks of death, myocardial infarction, hospitalization for congestive heart failure and stroke without improvements in quality of life [7]. These observations raise the possibility that EPO may have adverse effects on the cardiovascular system. Vascular smooth muscle and endothelial cells express both EPO and the EPO receptor (EPOR) [8]. Surprisingly, in spite of its widespread use in a population that is at high risk of cardiovascular morbidity and mortality, there is a dearth of mechanistic studies that address the effect of EPO on the cardiovascular system. A few studies, mostly conducted by the group of Vaziri, have revealed that EPO may contribute to the development of vascular disease in CKD by reducing NO bioavailability [9]. We and others have recently observed that EPO reduces the expression of NOS in endothelial cells (ECs) [9,10]. Moreover, EPO upregulated the expression of monocyte-chemoattractant protein-1 (MCP-1), a member of the C–C or beta subfamily of chemokines that is pivotal to the recruitment, adhesion and transendothelial migration of monocytes during atherogenesis. NO released from a series of synthetic donor compounds suppressed the EPO-mediated augmentation of cytokine-induced MCP-1 expression, suggesting that upregulation of MCP-1 expression is linked to EPO-induced downregulation of ecNOS [10]. Chan et al. have previously reported that monocytes cocultured with ADMA-exposed ECs are hyperadhesive [11]. ADMa also stimulated MCP-1 formation [12]. Hence, in the current study we sought to investigate the effect of EPO on dimethylarginine (DMA) elaboration by ECs and on serum DMA concentrations in Balb/c mice.

**Methods**

Recombinant human erythropoietin (Epogen) was obtained from Amgen (Thousand Oaks, CA, USA). HUVECs, aortic vascular smooth muscle cells (SMCs) and media (EGM-2 and SGM-2) were purchased from Clonetics (Walkersville, MD, USA). Colorectal adenocarcinoma (DLD-1) cells were obtained from American Type Culture Collection (Manassas, VA, USA).

**Cell culture**

HUVECs, SMCs and DLD-1 cells were grown as previously described [9]. DLD-1 cells were grown in DMEM supplemented with FBS (10%) and penicillin G sodium (100 U/ml), streptomycin sulfate (100 µg/ml) and amphotericin B (0.25 µg/ml). The L-arginine concentrations in the basal HUVEC and DLD-1 media were 0.17 mM and 0.38 mM, respectively. HUVECs and SMCs were used between first and third passage.

**Treatment of HUVECs, SMCs and DLD-1 cells**

Confluent HUVEC monolayers were exposed to medium or the indicated concentration of EPO, respectively, for 24 h. DLD-1 cells were exposed to medium or EPO (5 U/ml) for 24 h and subsequently incubated with the following cytokine cocktail for 48 h: 100 U/ml interferon-gamma (IFN-γ), 10 ng/ml tumor necrosis factor-alpha (TNF-α); 0.5 ng/ml interleukin-1beta (IL-1β) (R&D Systems, Minneapolis, MN, USA). Aliquots of the conditioned media were immediately analyzed by Griess assay. The remainders were frozen at −80°C for later quantitation of dimethylated arginine derivatives. Cell lysates were frozen at −80°C for iNOS or DDAH western blots. A fraction of the conditioned media was also subjected to cytotoxicity detection (LDH) assays (Roche Applied Science, Indianapolis, IN, USA) to assess cell viability. Cell viability was greater than 95%.

**Mice**

Experiments were conducted in accordance with the NIH guidelines for the care and use of laboratory animals. Male Balb/c mice, aged 12 weeks, were purchased from Harlan (Indianapolis, IN, USA), housed at the University of Michigan Unit for Laboratory Medicine (ULAM), subjected to fixed 12-h light/dark cycles and received an adjusted calories diet (TD88137, Harlan Teklad, Madison, WI, USA) and water ad libitum. Starting at the age of 13 weeks, the mice were injected subcutaneously (s.c.) with 30 U of Epogen every other day for 10 weeks. Control mice were injected s.c. with an equivalent volume (200 µl) of saline. The mice were euthanized by an i.p. injection of pentobarbital. Harvested organs and plasma were snap-frozen in liquid nitrogen.

**Blood pressure measurements**

Systolic blood pressure measurements were performed non-invasively using the tail-cuff method described by Whitesall et al. [13].

**Haematocrit determinations**

Haematocrit determinations were performed by the University of Michigan Unit for Laboratory Animal Medicine (ULAM) Diagnostic Laboratory by standard methods.

**Western blotting**

Following the removal of conditioned medium, HUVECs were rinsed with PBS and harvested in ice-cold radioimmunoprecipitation (RIPA) lysis buffer. To prepare whole kidney and liver lysates, tissue was homogenized in RIPA buffer at 4°C. Protein determination was carried out by Micro BCA assay (Pierce, Rockford, IL, USA). Western blots were performed as previously described [10]. Primary antibodies for detection of NOS or DDAH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at a dilution of 1:500. Blots were stripped by incubating in stripping buffer (Pierce, Rockford, IL, USA) and were reprobed with an antibody to β-actin (Sigma, St. Louis, MS, USA).

**DDAH enzyme activity assay**

Enzymatic activity of DDAH was measured as described by Stühlinger et al. [14].
Measurement of iNOS gene expression in DLD-1 cells

Total cellular RNA was extracted with Trizol (Sigma). A total of 1 µg of RNA was used. Messenger RNA (mRNA) transcripts were reverse transcribed by including Avian Myeloblastosis Virus Reverse Transcriptase (Boehringer, Mannheim, Germany) in the PCR reaction mixture. Complementary DNA (cDNA) products were PCR-amplified by including Taq DNA Polymerase (Boehringer). In the control reaction, reverse transcriptase was omitted. Primers were designed from published sequences deposited in the GenBank database and found by using a primer analysis software program (OLIGO; National Bioscience, Inc., Plymouth, MN, USA). They were as follows: iNOS: 5'-GGG AGC ATC ACC CCC GTG TT-3', 5'-GAG CGA TTT CTT CAG TTT CTC T-3'. The PCR product length of iNOS was 419 bp. PCR amplification was conducted through 35 cycles of denaturation at 94°C for 30 s, oligonucleotide annealing at 62°C for 1 min and extension at 72°C for 1 min. Reactions were electrophoresed on 3% agarose gels containing ethidium bromide in 0.5% TBE buffer to visualize the PCR products.

Densitometric scanning

Autoradiographs were scanned and integrated densities were determined as described [10]. To adjust for differences in sample loading between wells in western blots, DDAH or NOS protein levels, respectively, were expressed as ratios of integrated densities for DDAH or NOS products to β-actin products.

Quantification of dimethylated arginine derivatives

ADMA and SDMA were quantified as previously described [15].

Determination of urinary nitrite and nitrate (NOx) concentrations

Mice were housed in metabolic cages and urine was collected over a 24-h period. Urinary NOx concentrations were determined by Griess assay using a kit from Assay Designs (Ann Arbor, MI, USA). Each sample was analyzed in duplicate.

Statistical analysis

Analysis was performed using Statistics for Windows, version 2.0. All values are expressed as means ± standard error. Data were analyzed using the t-test or analysis of variance (ANOVA) with Bonferroni comparison of means, where appropriate. Probability (P) values of <0.05 were considered significant.

Results

Recombinant human EPO significantly suppresses ADMA and SDMA concentrations in HUVECs

We treated HUVECs with EPO or medium, respectively, for 24 h and measured the concentrations of ADMA and SDMA in a conditioned medium. As depicted in Figure 1A, ADMA concentrations in the conditioned media of HUVEC monolayers exposed to EPO were significantly lower than those in media-treated controls. Treatment of HUVECs with 5 U/ml of EPO resulted in a 15% decrease in ADMA concentrations in the conditioned medium whereas exposure to 50 U/ml of EPO reduced ADMA concentrations by 24% (Figure 1A). Exposure to 5 U/ml of EPO also resulted in a 36% decrease in SDMA concentrations in the conditioned medium and incubation with 50 U/ml of EPO reduced SDMA concentrations by 40% (Figure 1B). Exposure to EPO did not significantly affect ADMA or SDMA elaboration by DLD-1 cells (data not shown). In SMCs, EPO treatment resulted in elevated ADMA concentrations but the differences were not statistically significant (data not shown).

EPO upregulates the expression of DDAH II in HUVECs

The expression of DDAH II protein concentrations in HUVEC monolayers exposed to medium or EPO (5 U/ml), respectively, was assessed by western blotting (Figure 2). In HUVECs incubated in the presence of EPO for 24 h,
DDAH II protein concentrations were increased by 39% over media-treated controls.

**EPO augments DDAH activity in HUVEC monolayers**

DDAH hydrolyzes ADMA, yielding citrulline and dimethylamine. We prepared whole cell lysates from ECs treated with either EPO (5 U/ml) or medium, respectively, and measured DDAH metabolic activity. DDAH enzymatic activity in EPO-treated HUVECs was increased by 38% over media-treated controls.

**Effect of EPO on iNOS expression and nitrite concentrations in DLD-1 cells**

To assess the effect of EPO on nitrite elaboration *in vitro* we chose DLD-1 cells. These cells express iNOS mRNA (Figure 3A) and protein (Figure 3B) upon exposure to a cocktail of cytokines and produce nitrite at concentrations that can be measured by Griess assay (Figure 3C). EPO treatment increased the protein expression of cytokine-inducible iNOS (Figure 3B). Concomitantly, nitrite concentrations in the conditioned medium of EPO-pretreated cells were increased by 58% compared with cells treated with the medium alone (Figure 3C) prior to cytokine stimulation. Protein expression of DDAH II was unchanged by EPO exposure (data not shown).

**EPO raises serum ADMA but not SDMA concentrations in Balb/c mice**

We injected Balb/c mice subcutaneously with either vehicle (saline) or EPO every other day (30 U) for 10 weeks. As depicted in Figure 4, serum ADMA concentrations in Balb/c mice treated with 30 U of EPO were 46% higher than in saline-treated controls ($P = 0.002$). However, serum concentrations of SDMA did not significantly differ in either group ($0.69 \pm 0.17 \mu M$ in EPO-treated mice versus $0.54 \pm 0.12 \mu M$ in controls).

**Effect of EPO on haematocrit**

Hematocrit in EPO-treated mice was significantly elevated when compared to saline treated control mice ($69.4\% \pm 2.2$ in EPO-treated animals versus $47.8\% \pm 1.6$ in saline-treated mice; $P = 0.0002$).

**Effect of EPO on systolic blood pressure**

We measured systolic blood pressure at intervals of 2 weeks for up to 6 weeks and again at the end of the study period. In mice receiving EPO, blood pressure was significantly elevated over controls after 2, 6 and 10 weeks of treatment (Figure 5).

**Effect of EPO on DDAH expression in the kidney**

Leiper *et al.* studied the tissue distribution of human DDAH II and observed that it was most highly expressed in heart, kidney and placenta [16]. We measured expression of DDAH II in the kidney of mice that had been treated with EPO for 10 weeks and in saline-treated control mice. EPO treatment did not have any effect on the protein expression of DDAH II in the kidneys of Balb/c mice. However, EPO did upregulate the expression of DDAH I, the dominant isoform in the kidney (Figure 6A).
EPO augments serum asymmetric dimethylarginine concentrations in mice

Effect of EPO on DDAH expression in the liver

In the livers of EPO-treated mice, DDAH I expression was upregulated by 18% when compared with saline-treated controls while protein expression of DDAH II was reduced by 29% (Figure 6B).

Effect of EPO on urinary NOx concentrations

We measured NOx concentrations in 24-h urine samples from EPO-treated and saline-injected control mice at 5 weeks and 10 weeks by Griess assay. At both time points, nitrite concentrations in the urine of EPO-treated mice were elevated over controls (Figure 7). However, only at 10 weeks was there a statistically significant difference in urinary NOx concentrations between saline- and EPO-treated mice.
Fig. 8. Expression of nitric oxide synthase in the kidneys of Balb/c mice treated with saline or EPO. Whole kidney lysates were analyzed by western blot using antibodies directed against NOS 1, NOS 2 or NOS 3. Each blot was stripped and reprobed with an antibody directed against β-actin. All integrated densities are displayed as ratios of NOS and β-actin protein products. Asterisk denotes a statistically significant difference from controls (saline). $n = 6$ for saline treatment and $n = 8$ for EPO treatment.

**EPO upregulates renal NOS expression**

To investigate whether the increased urinary NOx concentrations following 10 weeks of EPO administration were accompanied by changes in renal NOS expression, we performed a series of western blot experiments. We observed that expression of NOS 1 was 52% higher in the kidneys of EPO-treated mice when compared with those of saline-treated controls. Expression of NOS 2 (iNOS) was 47.9% higher in EPO-treated mice when compared with saline-treated controls. Expression of NOS 3 in the kidney was also elevated, but the difference was not statistically significant (Figure 8).

**Discussion**

In patients with CKD, serum concentrations of ADMA are elevated and correlate with vascular disease and cardiovascular mortality [3,6]. These patients are also in an NO-deficient state suggesting a link between elevated ADMA concentrations and reduced NO bioavailability [2]. Currently available *in vitro* data and clinical evidence suggest that even small modifications of circulating ADMA levels significantly change vascular NO production, vascular tone and systemic vascular resistance [3]. ADMA and SDMA are synthesized when arginine residues in proteins are posttranslationally methylated by the action of protein arginine methyltransferases (PRMTs). After proteolysis of arginine-methylated proteins, free $N^{G}$-monomethyl-L-arginine (L-NMMA), ADMA and SDMA are released in the cytosol. It was previously assumed that ADMA was mainly eliminated via renal excretion. However, it is now widely accepted that DDAH may be a significant route of elimination for ADMA but not for SDMA. DDAH that exists in two major isoforms (DDAH I and DDAH II) in humans, is found in virtually every cell type and metabolizes ADMA via hydrolysis, yielding citrulline and dimethylamine. Of the total ADMA produced daily in humans, only 20% is excreted by the kidneys whereas the remaining 80% is metabolized by DDAH. Dysregulation of DDAH with consecutive increases in plasma ADMA concentrations and chronic NOS inhibition has therefore been proposed as a common pathophysiological pathway under various clinical conditions [3,4,6]. It is currently unknown
which DDAH isoforms or which organ dominates ADMA degradation in humans. Based on studies performed in the rat, kidney, liver and endothelium are hypothesized to be the major organs that metabolize ADMA, and renal DDAH is thought to be a key player in the renal extraction of ADMA. In endothelium, DDAH II is the major isoform, whereas in kidney DDAH I is the principal isoform.

We have recently observed that EPO reduces both endothelial nitric oxide synthase (eNOS) expression and enzymatic activity in HUVECs [10]. Furthermore, EPO upregulates the expression of MCP-1, a pivotal mediator of atherogenesis [10]. In the current study, we investigated the effect of EPO on DMA elaboration by cultured ECs and on DMA serum concentrations in Balb/c mice. Contrary to our hypothesis, we observed that at therapeutically relevant concentrations, EPO suppressed elaboration of ADMA and its biologically inactive stereoisomer symmetric dimethylarginine (SDMA) by HUVECs. Furthermore, EPO upregulated the expression as well as the metabolic activity of DDAH II in vitro. Since EPO reduced both ADMA and SDMA concentrations in HUVECs in vitro, elevation of DDAH expression and activity cannot account for this observation. In this regard, our in vitro data lead us to speculate that EPO may reduce either PRMT-mediated synthesis of DMAs or the proteolysis of arginine-methylated proteins.

Following completion of these in vitro experiments, but while our in vivo studies were still in progress, Scalera et al. reported that EPO (at concentrations ranging from 10 to 200 U/ml) increases ADMA concentrations in ECs [17]. Concomitantly, DDAH activity was reduced. At lower doses (i.e. 0.1 and 1 U/ml) of EPO, no effect on ADMA concentrations was noted. In our experiments on the other hand, EPO at 5 U/ml significantly reduced ADMA concentrations in vitro. While the reasons for the conflicting results from our study and that of Scalera et al. cannot be pinpointed with absolute certainty, it must be noted that the doses of EPO at which these researchers reported augmentation of ADMA concentrations may not be physiologically relevant. Physiological concentrations of EPO in most healthy animals and humans are in the range of 4–30 mU/ml, but up to 100-fold increases can be seen in severe anemias (thus resulting in plasma concentrations as high as 3 U/ml) [18]. Furthermore, relatively high EPO plasma concentrations in the order of 1–5 U/ml may be obtained intermittently in patients immediately after intravenous injection of the hormone. Additional differences between the two studies are the assessment of SDMA concentrations in conditioned media from HUVEC monolayers in our study and the NOx measurements in the paper published by Scalera et al. However, similar results in both studies were obtained in vivo. We found that in healthy Balb/c mice a 10-week course of EPO results in a significant rise in serum ADMA concentrations. This observation supports the preliminary finding published by Scalera et al. who reported a 16% rise in ADMA plasma concentrations in patients with CRD who received EPO for 7 days.

The discrepancy between our in vivo and in vitro observations underscore the difference in the effect that EPO may exert on isolated ECs versus effects seen at the level of the whole animal. Conversely, the seemingly conflicting in vitro and in vivo data presented in our study may simply highlight the multifaceted role that EPO plays in the cardiovascular system and the complex pathways involved in generation and degradation of ADMA. Although EPO may reduce ADMA concentrations in ECs even in the whole animal, circulating ADMA concentrations may be elevated due to increased ADMA generation and/or decreased ADMA metabolism in other tissues or cell types. In this regard, little is known at present regarding the metabolism of ADMA. Billecke et al. recently observed that rat erythrocytes possess significant DDAH activity and also constitute a large reservoir of protein-incorporated ADMA [19]. Pathological conditions characterized by red blood cell fragility or enhanced expression of proteolytic enzymes may therefore facilitate increases in plasma ADMA concentrations. A correlation between ADMA and erythrocyte fragility in hypertensive subjects has been reported. Although we are unsure whether the same holds true for murine erythrocytes, the data presented by Billecke et al. may be relevant to our study since EPO treatment results in a marked elevation in haematocrit values (from 47.8% ± 1.6% in saline-treated controls to 69.4% ± 2.2% in EPO-treated Balb/c mice, P = 0.0002). It is conceivable that the EPO-mediated increase in the number of erythrocytes may result in greater quantities of stored ADMA in the blood. Additionally, the EPO-mediated increases in blood pressure may render these erythrocytes more fragile allowing them to release ADMA into the plasma.

Our study also reveals some differences in the in vitro and in vivo effect of EPO on DDAH expression. The expression of DDAH II was increased in vitro but not significantly different in the kidneys of Balb/c mice. However, the expression of DDAH I, the major isoform in the kidney, was upregulated in Balb/c mice following 10 weeks of EPO administration. In the livers of EPO-treated mice, DDAH I expression was increased while DDAH II protein concentrations were significantly lower than those in the livers of saline-treated control mice. Since serum ADMA but not serum SDMA concentrations were elevated following 10 weeks of EPO administration, it is likely that EPO-mediated effects on DDAH rather than on protein catabolism and/or PRMT activity are responsible for the rise in serum ADMA concentrations.

In 2005, Kielstein and Zoccali counted only six animal studies regarding the effect of ADMA in vivo [20]. We found less than a handful of studies documenting the normal plasma concentrations of ADMA in wild-type rats or mice and were unable to find any published study in which both serum ADMA and urinary NOx concentrations were simultaneously assessed in mice. To the best of our knowledge, this is the first study aimed at unraveling the effect of long-term EPO therapy on serum ADMA concentrations in an animal model and on systemic NO production in mice. Interestingly, in our experiments the rise in ADMA did not result in NO deficiency in EPO-treated mice. This observation is in agreement with reports from several laboratories that have studied the effect of short-term EPO administration on NO production in rats. Tsukahara et al. reported that a 2-week treatment of Sprague Dawley rats with EPO resulted in increased urinary nitrate and nitrite excretion when compared with controls [21]. Co-administration of L-NAME, an inhibitor of the constitutive form of NOS,
reversed the effect of EPO on NO excretion. Based on these observations it was concluded that the pressor effect of EPO is not due to an NO-depleting effect. Kanagy et al. also documented an increase in plasma NOx concentrations following 2 weeks of EPO administration to rats [22]. This was accompanied by an increase in the aortic expression of NOS 3. The researchers failed to detect a significant upregulation of NOS 3 in the kidney of EPO-treated rats when compared with those of controls. Similar to these experiments in which rodents were treated with exogenous EPO, transgenic mice overexpressing EPO also exhibited enhanced circulating NO bioavailability [23].

As opposed to the short-term studies with EPO performed in rats, our data reveal that long-term treatment of healthy Balb/c mice with EPO also results in significant augmentation of endogenous NO production and induces profound changes in the expression of NOS in the kidney. Similar to Kanagy et al., we did not observe any change in NOS 3 expression in the kidney [22]. The novel observation in our study is that NOS 1 and NOS 2 expression in the kidneys of Balb/c mice is significantly elevated following 10 weeks of EPO administration. Taken collectively with the observations of Tsukahara et al. and Kanagy et al. our data suggest that chronic EPO treatment induces a compensatory increase in NO release, perhaps in part due to upregulation of NOS 1 and NOS 2 expression in the kidney [21,22]. The EPO-induced rise in serum ADMA concentrations does not prevent this compensatory increase in NO release. Although our study does not provide any mechanistic data to explain the apparent discrepancy between elevated serum ADMA concentrations and increased urinary output of nitrite and nitrate, it is conceivable that the EPO-mediated elevation in ADMA concentrations is simply insufficient to exert an effect on NOS. In this regard it is known that the IC50 for NOS inhibition by ADMA is dependent on prevailing arginine concentrations. However, since our current understanding of ADMA metabolism in mice and men is extremely limited, further studies are clearly necessary to discern whether our observations in mice apply to humans receiving EPO therapy. Such studies would be of particular relevance to the care of anaemic patients with CRD in whom NO bioavailability may already be compromised [2].

Acknowledgements. This research was supported by scientist development grant N004317 from the American Heart Association Midwest Affiliate to Anjali Desai, Ph.D., M.S. We gratefully acknowledge the excellent technical assistance of Robin Kunkel and Elizabeth Walker in the preparation of the figures. We thank the laboratory of Dr. Lou G. D’Alessy for determining DMA concentrations. We are also indebted to Janet Hoff for her assistance with blood pressure measurements.

Conflict of interest statement. None declared.

References


Received for publication: 22.1.07
Accepted in revised form: 15.11.07