Soluble vascular endothelial growth factor receptor-1 is reduced in patients with resistant hypertension after renal denervation

Running title: Reduction in sVEGFR-1 post RDN

Nina Eikelis[a] PhD, Dagmara Hering[a,b] MD, PhD, Petra Marusic[a,b] BSc, Antony S. Walton[c] MD, PhD, Elisabeth A. Lambert[a] PhD, Yusuke Sata[a] MD, PhD, Henry Krum[d*] MD,PhD, Gavin W Lambert[a] PhD, Murray D. Esler[a,b] MD, PhD, Markus P. Schlaich[a,b,c] MD, PhD

[a]Neurovascular Hypertension & Kidney Disease Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Australia; [b]School of Medicine and Pharmacology - Royal Perth Hospital Unit, University of Western Autralia, Perth, Australia; [c]Heart Centre Alfred Hospital, Melbourne, Australia; [d]Monash Centre of Cardiovascular Research and Education in Therapeutics, School of Public Health & Preventive Medicine, Monash University, Melbourne Australia. * author deceased

Source of support: The research described in this paper has been partly supported by the National Health and Medical Research Council of Australia and the Victorian Government's Operational Infrastructure Support Program.

Address for correspondence and reprint requests should be directed to:

Dr Nina Eikelis

Baker IDI Heart and Diabetes Institute

PO Box 6492, St Kilda Road Central

Victoria, 8008

Australia

Phone: +61 8532 1348

Fax: +61 8532 1100

Email: nina.eikelis@bakeridi.edu.au

Abstract

Renal denervation (RDN) has been shown in several studies to reduce blood pressure (BP) in patients with resistant hypertension (RH). Data on potential biomarkers associated with BP changes remain scarce. We evaluated whether soluble vascular endothelial growth factor receptor (sVEGFR-1) is affected by the procedure**.** A total of 57 patients with RH participated in this study. BP and heart rate were recorded at baseline and 3 months follow up, at which time blood samples were collected to determine the levels of sVEGFR-1, VEGF-A, VEGF-C, nitric oxide (NO), sVCAM-1 and sICAM-1. None of the biomarkers had a predictive value that could identify responders vs non-responders to RDN. However, sVEGFR-1 concentration was dramatically reduced after RDN (5913±385 vs 280±57 pg/ml, $p<0.001$). At the same time VEGF-A levels were significantly increased (10.0±3.0 vs 55.5±7.9 pg/ml, $p<0.001$), without significant changes in VEGF-C. NO levels were significantly increased after RDN in the whole group (82.6±6.2 vs 106.9±7.8 uU, $p<0.001$). Interestingly, the elevation in NO levels at 3 months was only seen in patients who demonstrated a reduction in systolic blood pressure of ≥ 10 mmHg (78.9±8.3 vs 111.6±11.7 uU, $p<0.001$). We report a significant reduction in sVEGFR-1 levels post RDN procedure which was accompanied by a significant increase in VEGF-A concentration as well as NO. Changes in plasma cytokines were not quantitatively linked to magnitude of BB reduction. An RDN-induced reduction in sVEGFR-1 plasma levels and increase in VEGF-A would raise the VEGF-A/sVEGFR-1 ratio, thereby increasing VEGF-A bioavailability to act on its full length receptor and may contribute to the BP lowering effect potentially via NO mediated pathways.

Introduction

Hypertension is a major risk factor for a coronary heart disease and stroke. About 10% of patients diagnosed with high blood pressure (BP) have resistant hypertension (RH), defined as high BP that remains uncontrolled despite treatment with at least three antihypertensive drugs (1). The activation of the sympathetic nervous system has been shown to play a major role in the aetiology and maintenance of raised BP (2). Over recent years a procedure based on the radiofrequency ablation of the renal sympathetic nerves has emerged as a potential treatment for patients with RH (3), although its efficacy in lowering BP has been questioned in a recent sham-controlled clinical trial (4).

While the BP lowering effects of renal denervation (RDN) has been demonstrated in a number of clinical trials (3, 5, 6) and smaller mechanistic studies (7-9), non-response to treatment (defined as a reduction in office systolic BP of less than 10 mmHg following RDN) is frequently reported in a subset of patients. The rates of this non-response to treatment are reported to range between 8-37% (10). Identification of biomarkers that can successfully predict BP reduction after RDN has therefore been a main focus of interest.

Dörr and colleagues (11) were the first to report on a number of potential predictive biomarkers, namely soluble intracellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1) and soluble vascular endothelial growth factor receptor 1 (sVEGFR-1). Their results indicated that responders (those patients that had a reduction in office systolic BP of at least 10 mmHg or more) had significantly higher levels of the aforementioned cytokines compared to non-responders, and as such postulated that the plasma levels of these compounds could potentially be used as biomarkers, predictive of successful BP reduction.

sVEGFR-1, also known as sFLT-1, is a soluble receptor for a vascular endothelial growth factor (VEGF). sVEGFR-1 binds VEGF thereby reducing its circulating levels, with sVEGFR-1 acting as a scavenger of circulating VEGF. Some studies suggest that VEGF may play a vascular protective role through its actions on nitric oxide (NO) (12). For example, it has been demonstrated that VEGF can stimulate e-NOS expression in cultured endothelial cells which results in increased production of bioactive NO (12).

Therefore, the aims of this study were two-fold, firstly, to assess the reproducibility of previous observations that sVEGFR-1, sICAM-1 and sVCAM-1 can be used as potential predictors of BP reduction after RDN; and secondly, to test if RDN-induced changes in the sVEGFR-1/VEGF ratio may lead to increased NO bioavailability as a potential mechanism of the BP lowering effects of RDN.

Methods

Patients

Fifty seven consecutive patients with RH, who were participating in our RDN program, formed the cohort for this study (5, 13). All participants underwent relevant biochemical and imaging studies to exclude secondary forms of hypertension. Hypertension was diagnosed according to the European Society of Hypertension guidelines for the management of arterial hypertension (14) and the current statement of the American Heart Association (15). The clinical definition of resistant hypertension included BP $\geq$ 140/90 mmHg despite treatment with at least three antihypertensive drugs, one of which was a diuretic. Clinical history was recorded for each participant.

Office seated BP was measured after 5 minutes of rest on both arms and was calculated as the average of three consecutive measurements within a 2-minute interval at baseline and 3 months following RDN using a validated semi-automatic oscillometric device (Omron HEM-907, Omron Healthcare Singapore PTE Ltd). The arm with higher BP readings was used for subsequent measures.

All participants provided written informed consent and the study was approved by the Alfred Hospital Ethics Committee. All investigations conformed to the principles outlined in the Declaration of Helsinki.

Renal denervation procedure

Renal denervation (RDN) was performed according to standard clinical practice with the Symplicity catheter (Medtronic). Details of the denervation procedure have been described previously (3, 5, 16).

Laboratory tests

Blood samples were drawn into EDTA containing chilled tubes at baseline and at the 3-month follow-up visit with patients in the supine position after ~30 minutes of rest for the determination of sVEGFR-1, VEGF-A, VEGF-C and nitric oxide (NO). sICAM-1 and sVCAM-1 levels were also measured. Blood samples were centrifuged and plasma was frozen at -80^{0}C until assayed.

sVEGFR-1, VEGF-A, VEGF-C, sICAM-1 and sVCAM-1 were measured with the use of enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. Sample absorbencies were recorded using a Benchmark Plus Microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA).

We measured nitrate and nitrite as an indicator of total NO production (17) with the use of the commercial colourimetric kit from Cayman Chemical Company (Ann Arbor, MI, USA). The final products of NO *in vivo* are nitrate (NO_3-) and nitrite (NO_2-), with the best index of NO production being the sum of both NO_3- and NO_2-. Measurement of total nitrate/nitrite concentration was achieved in a two-step process. The first step involved the conversion of nitrate to nitrite using nitrite reductase. In the second step, addition of the Griess Reagent resulted in the conversion of nitrite into a deep purple compound, which was photometrically measured using Benchmark Plus Microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA). Griess reaction is the most frequently used analytical method for quantification of the major metabolites of NO (NO_3- and NO_2-) in blood (17).

For all laboratory tests the samples were assayed in duplicates. Quality controls were included in each run to check for inter-assay variability. For each patient baseline and follow up samples were measured within the same assay. The person doing the assays was naive to the blood pressure response.

Statistical Analysis

Statistical analyses were performed using SigmaStat for Windows Version 3.5 (Jandel Scientific, San Rafael, CA). Two way repeated measures ANOVA was used to compare the differences between BSL and follow up in the whole group as well as in responders vs non-responders. Univariate correlation between the reduction in BP and physiological and experimental variables were evaluated using Pearson's (for normally distributed variables) or Spearman's (for not normally distributed variables) correlation coefficient in the overall group and then on both the responders and non-responders. Data are expressed as mean±SEM. Statistical significance was set at $p<0.05$

Results

Characteristics of the participants are presented in Table 1. Patients who demonstrated a reduction in systolic blood pressure (SBP) of ≥10 mmHg at 3 months follow up were defined as responders, while those with a SBP reduction of <10mmHg were defined as non-responders. Participants were prescribed an average of 4.6±0.2 antihypertensive medications. The mean office SBP was 168.8±2.6 mmHg. At 3-month follow up visit office SBP was significantly reduced to 155.5±3.2 ($p<0.001$). The same was true for the office diastolic blood pressure (DBP) (90.7±2.3 vs 83.7±2.4 mmHg, $p<0.001$). There was no effect of RDN on heart rate.

Thirty one patients (54% of the total cohort) demonstrated a reduction in SBP of ≥10 mmHg at 3 months post denervation. There were no differences in baseline SBP or DBP between responders and non-responders (Table 1). Non-responders had a significantly higher heart rate at rest (73.4±3.5 vs 63.9±3.3 beats/min, $p=0.020$).

In the entire group of 57 patients we observed significant increases in sVCAM-1 levels after RDN (685.5±19.7 vs 738.2±23.0 ng/ml, $p<0.001$). This increase was evident in both the responder group (691.4±25.9 vs 748.0±27.9 ng/ml, $p=0.006$) and non-responder group (678.5±30.8 vs 726.4±38.5 ng/ml, $p=0.033$) (Table 2). The reduction in SBP was not associated with baseline levels of sVCAM-1 either in the whole group or sub-group analyses.

There was a small but significant increase in sICAM-1 concentrations before and after RDN in the whole group (209.1±5.9 vs 221.0± 7.7 ng/ml, $p=0.039$). However, the differences were not present in subgroup analysis. The reduction is SBP was not associated with baseline levels of the cytokine either in a whole group or sub-group analyses.

The concentrations of sVEGFR-1, VEGF-A and VEGF-C are presented in Figure 1 and Table 2. Measurements of sVEGFR-1 revealed significantly higher levels of sVEGFR-1 concentrations at baseline (5913.9±384.8 vs 279.8±57.2 pg/ml, $p<0.001$). No significant differences in baseline sVEGFR-1 were detected between responders and non-responders, with reductions in sVEGFR-1 after RDN being comparable between groups.

Plasma VEGF-A was significantly higher after RDN in the whole group (10.0±3.0 vs 55.5±7.9 pg/ml, $p<0.001$). The increase in VEGF-A was similar between responders and non-responders and there were no differences in baseline levels between the groups (Table 2, figure 1). There was no association between the fall in SBP and baseline levels of VEGF-A. We have also measured the levels of VEGF-C which, unlike VEGF-A, does not bind to sVEGFR-1. There were no differences in VEGF-C levels before and after treatment either in a whole group or subgroups and no associations with SBP fall.

Measurements of NO (total of nitrate and nitrite) revealed significantly higher levels at 3 month post procedure (82.6±6.2 vs 106.9±7.8 uM, $p<0.001$) in the whole group (Figure 2). However, this increase was only seen in responders (78.9±8.3 vs 111.6 ±11.7 uM, $p<0.001$) (Figure 2). There were no associations between baseline NO levels and the reduction in SBP.

Discussion

Reaching blood pressure goals is an important aspect in treating patients with hypertension, however, in some cases it is very difficult to achieve. Given the lack of adherence to anti-hypertensive medications, there has been growing interest in device-based therapeutic therapies such as sympathetic nerve ablation. The Symplicity HTN-1 and 2 trials, as well as smaller uncontrolled studies, demonstrated a significant reduction in systolic blood pressure following

renal denervation. More importantly that reduction has been shown to be sustained up to at least 3 years follow-up (3, 5). The Symplicity HTN-3 trial, however, raised some concerns over the effectiveness of the procedure (4). In addition, it has been shown, that not all patients benefit from renal denervation. Therefore, it is important to identify a biomarker(s) that could predict treatment response. In this study we measured a number of cytokines, previously shown to have a predictive value in identifying responders vs non-responders (11). None of the plasma cytokines measured in this study (sVCAM-1, sICAM-1, sVEGFR-1, VEGF-A or VEGF-C) were correlated with the reduction in systolic blood pressure at 3 months post procedure. Interestingly, while baseline cytokine concentrations were not different between responders and non-responders, we noted significant changes in the levels of sVEGFR-1 and VEGF-A at 3 months post procedure.

VEGF is a potent mitogen for vascular endothelial cells (18). Although there are multiple variants of VEGF, the angiogenic effects are mainly mediated through its most common variant, VEGF-A (often referred to simply as VEGF). VEGF-A exerts its effects via two high affinity receptors: sVEGFR-1 and VEGFR-2 with the latter receptor being the major mediator of its actions (19). sVEGFR-1 (also known as sFlt-1) is a splice variant of the vascular endothelial growth factor receptor that lacks the cytoplasmic and transmembrane domains but has an intact ligand binding site. It binds VEGF-A with the same efficiency as a functional VEGFR-2 receptor (also known as Flk-1) capable of transmitting a signal. Therefore, sVEGFR-1 acts as an endogenous inhibitor of VEGF-A. Scavenging of VEGF-A elevates BP in patients receiving anti-angiogenic therapy. Similarly, inhibition of circulating VEGF by its soluble receptor underlies BP elevation (20).

The major finding of the current study is a highly significant reduction in sVEGFR-1 levels post RDN. Reduction in circulating sVEGFR-1 was documented in all but two patients but was independent of changes in office BP at 3 months after RDN. It should be noted, however, that baseline plasma concentrations of sVEGFR-1 reported in our study are substantially higher than those described in a previously published paper (11). In particular, Dörr *et al* (11) report sVEGFR-1 values between 100-150 pg/ml, whereas in our study the concentrations at the baseline visit were around 5,500 pg/ml. These differences are unlikely due to different methodologies used as the same commercial kit was employed in both studies. It is unclear why there is such a disparity in the values given the patient population was similar between the studies. The levels seen at a follow up visit are about two times higher than in the Dörr study, with the only difference being that sVEGFR-1 was measured at 6 months (11) rather than 3 months follow up. Irrespective of this discrepancy, the reduction in sVEGFR-1 from baseline was highly significant in our study and reproducible.

High sVEGFR-1 levels are commonly seen in patients with preeclampsia (21). Elevated levels have been also reported in patients with chronic kidney disease which was associated with decreased renal function (22). Another study reported increased sVEGFR-1 levels in a cohort of essential hypertensive patients (23). A number of reports indicate that heparin leads to increased plasma levels of sVEGFR-1 (24, 25). In this view, it must be stated that all patients had a standard dose of heparin on the day of the procedure when the baseline sample was drawn, which potentially resulted in increased sVEGFR-1 concentration. However, heparin was also administered to 34 patients on the follow up visit. While those that received heparin at follow up visit did have higher sVEGFR-1 levels compared to those that did not (352.4±85.8 vs 149.8±27.0 pg/ml, p=0.312, data not shown), the reduction in circulating sVEGFR-1 levels was very

significant in both groups, irrespective of heparin administration. Taken together these observations suggest that, while heparin most likely resulted in higher circulating sVEGFR-1 concentration, there was still a significant reduction after renal ablation.

In variance with the data presented by Dörr and colleagues (11) is our lack of observation that baseline sVEGFR-1 concentration was predictive of BP fall after nerve ablation. We have previously reported the levels of sICAM-1 and sVCAM-1 prior and following denervation (13). In the present paper the baseline levels of these cytokines have been examined with a view of determining whether their concentrations before the procedure could predict a successful denervation as demonstrated by Dörr *et al* (11). Similarly to sVEGFR-1 there was no correlation between baseline sVCAM-1 and sICAM-1 and SBP or change in SBP at a follow up visit. However, unlike sVEGFR-1, observed concentrations of sVCAM-1 and sICAM-1 in the current paper are similar to those reported by Dörr *et al* (11).

Interestingly, we also report a significant increase in circulating levels of VEGF-A after denervation. VEGF-A plays a role in numerous physiological processes, including stimulation of blood vessel formation. Such VEGF-A induced angiogenesis has been implicated in cancer and other human diseases. To slow down the progression of cancerous tumours, VEGF inhibitors have been developed, and while some have shown promise in clinical trials, they are frequently associated with side effects, in particular elevation in BP. In fact, a series of experimental studies have provided further support for significant BP lowering effects of VEGF-A (26, 27). Knocking down sVEGFR-1 resulted in an increased production/action of VEGF, which in turn stimulated NO release (28). Importantly, in the current study, a reduction in sVEGFR-1 concentration was associated with a significant increase in NO levels, as assessed by established methodology (17).

Earlier studies by Tsurumi and colleagues (29) suggested an interaction between VEGF and NO production showing that VEGF released from a damaged vessel wall can stimulate re-endotheliasation by acting in concert with NO. VEGF has been shown to induce vasodilatation *in vitro* as a result of endothelial derived NO (30). Furthermore, other studies also showed that VEGF can stimulate eNOS-derived NO production (31). Reduced expression of nitric oxide synthase enzymes (eNOS and nNOS) has been demonstrated in the kidneys of mice undergoing anti-VEGFR-2 antibody treatment (26). Taken together these data suggest that chronic inhibition of a functional VEGF receptor (VEGFR-2) may reduce NO production which in turn contributes to BP elevation.

An interesting observation from our study is that NO elevation after RDN was only seen in responders. There was no significant change in NO concentration in patients that demonstrated a reduction in office SBP of less than 10 mmHg at 3 months after RDN, despite a significant drop in sVEGFR-1 and coincident increase in VEGF-A concentrations. A possible explanation not tested in this study may be uncoupling of the VEGF-NO pathway that prevents generation of NO despite excess abundance of VEGF-A, thereby limiting the BP lowering effect. While theoretically possible, this is clearly a hypothesis that needs to be tested in adequate molecular experiments.

In summary, our study documents a marked reduction in sVEGFR-1 and a concomitant increase in VEGF-A. A significant increase in NO levels however, was only evident in those patients who experienced a clinically relevant BP reduction. None of the plasma cytokines measured had a predictive value in terms of differentiating reponders vs non-responders. We conclude that an RDN-induced reduction in sVEGFR-1 plasma would raise VEGF-A bioavailability to act on its full length receptor and contribute to the BP lowering effect via NO mediated pathways.

Summary table

What is known about this topic:

- Prevalence of RH is 10-20% in the general hypertensive population.
- Renal nerve ablation leads to a significant reduction in blood pressure in patients with RH.

What this study adds:

- This is the first study to demonstrate significant changes in plasma levels of sVEGFR-1, VEGF-A and NO in patients with resistant hypertension 3 months after renal denervation.
- This study demonstrates that blood pressure reduction, following RDN, is at least to some degree attributable to increased VEGF-A bioavailability, potentially via increased NO mediated pathways.

Acknowledgments

We wish to thank the study participants for their time, cooperation and effort.

Conflict of interest

Drs Walton, Krum, G Lambert, Esler and Schlaich are investigators in studies sponsored by Medtronic. The laboratories of Drs Schlaich, G Lambert and Esler currently received research funding from Medtronic. Professor G Lambert has acted as a consultant for Medtronic and has received honoraria or travel support for presentations from Pfizer, Wyeth Pharmaceuticals, Servier and Medtronic. Professor Esler serves on scientific advisory boards of Abbott (formerly Solvay) Pharmaceuticals and Medtronic. Professor Schlaich serves on scientific advisory boards for Abbott (formerly Solvay) Pharmaceuticals, BI, Novartis Pharmaceuticals, and Medtronic and has received honoraria and travel support from Abbott, BI, Servier, Novartis, and Medtronic.

References

1. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.

2. Esler M, Kaye D. Sympathetic nervous system activation in essential hypertension, cardiac failure and psychosomatic heart disease. J Cardiovasc Pharmacol. 2000;35((7 Suppl 4)):S1-7.

3. Symplicity HTNI, Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. Lancet. 2010;376(9756):1903-9.

4. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med. 2014;370(15):1393-401.

5. Krum H, Schlaich M, Whitbourn R, Sobotka PA, Sadowski J, Bartus K, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet. 2009;373(9671):1275-81.

6. Krum H, Schlaich MP, Sobotka PA, Bohm M, Mahfoud F, Rocha-Singh K, et al. Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. Lancet. 2014;383(9917):622-9.

7. Hering D, Lambert EA, Marusic P, Walton AS, Krum H, Lambert GW, et al. Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. Hypertension. 2013;61(2):457-64.

8. Hering D, Marusic P, Walton AS, Lambert EA, Krum H, Narkiewicz K, et al. Sustained Sympathetic and Blood Pressure Reduction 1 Year After Renal Denervation in Patients With Resistant Hypertension. Hypertension. 2014;64(1):118-24.

9. Azizi M, Sapoval M, Gosse P, Monge M, Bobrie G, Delsart P, et al. Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. Lancet. 2015;385(9981):1957-65.

10. Esler MD, Krum H, Schlaich M, Schmieder RE, Bohm M, Sobotka PA, et al. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. Circulation. 2012;126(25):2976-82.

11. Dörr O, Liebetrau C, Mollmann H, Gaede L, Troidl C, Rixe J, et al. Soluble fms-Like Tyrosine Kinase-1 and Endothelial Adhesion Molecules (Intercellular Cell Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1) as Predictive Markers for Blood Pressure Reduction After Renal Sympathetic Denervation. Hypertension. 2014;63(5):984-90.

12. Bouloumie A, Schini-Kerth VB, Busse R. Vascular endothelial growth factor up-regulates nitric oxide synthase expression in endothelial cells. Cardiovasc Res. 1999;41(3):773-80.

13. Eikelis N, Hering D, Marusic P, Sari C, Walton A, Phillips S, et al. The effect of renal denervation on endothelial function and inflammatory markers in patients with resistant hypertension. Int J Cardiol. 2015;188:96-8.

14. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management

of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2007;25(6):1105-87.

15. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Hypertension. 2008;51(6):1403-19.

16. Schlaich MP, Sobotka PA, Krum H, Lambert E, Esler MD. Renal sympathetic-nerve ablation for uncontrolled hypertension. N Engl J Med. 2009;361(9):932-4.

17. Tsikas D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2007;851(1-2):51-70.

18. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246(4935):1306-9.

19. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem. 1998;273(46):30336-43.

20. Amraoui F, Spijkers L, Hassani Lahsinoui H, Vogt L, van der Post J, Peters S, et al. SFlt-1 elevates blood pressure by augmenting endothelin-1-mediated vasoconstriction in mice. PLoS One. 2014;9(3):e91897.

21. Hertig A, Berkane N, Lefevre G, Toumi K, Marti HP, Capeau J, et al. Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia. Clin Chem. 2004;50(9):1702-3.

22. Di Marco GS, Kentrup D, Reuter S, Mayer AB, Golle L, Tiemann K, et al. Soluble Flt-1 links microvascular disease with heart failure in CKD. Basic Res Cardiol. 2015;110(3):30.

23. Belgore FM, Blann AD, Li-Saw-Hee FL, Beevers DG, Lip GY. Plasma levels of vascular endothelial growth factor and its soluble receptor (SFlt-1) in essential hypertension. Am J Cardiol. 2001;87(6):805-7, A9.

24. Hagmann H, Bossung V, Belaidi AA, Fridman A, Karumanchi SA, Thadhani R, et al. Low-molecular weight heparin increases circulating sFlt-1 levels and enhances urinary elimination. PLoS One. 2014;9(1):e85258.

25. Rosenberg VA, Buhimschi IA, Lockwood CJ, Paidas MJ, Dulay AT, Ramma W, et al. Heparin elevates circulating soluble fms-like tyrosine kinase-1 immunoreactivity in pregnant women receiving anticoagulation therapy. Circulation. 2011;124(23):2543-53.

26. Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM. Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. Hypertension. 2009;54(3):652-8.

27. Li Z, Zhang Y, Ying Ma J, Kapoun AM, Shao Q, Kerr I, et al. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. Hypertension. 2007;50(4):686-92.

28. Ahmad S, Hewett PW, Al-Ani B, Sissaoui S, Fujisawa T, Cudmore MJ, et al. Autocrine activity of soluble Flt-1 controls endothelial cell function and angiogenesis. Vascular cell. 2011;3(1):15.

29. Tsurumi Y, Murohara T, Krasinski K, Chen D, Witzenbichler B, Kearney M, et al. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. Nat Med. 1997;3(8):879-86.

30. Ku DD, Zaleski JK, Liu S, Brock TA. Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries. Am J Physiol. 1993;265(2 Pt 2):H586-92.

31. Morales-Ruiz M, Fulton D, Sowa G, Languino LR, Fujio Y, Walsh K, et al. Vascular endothelial growth factor-stimulated actin reorganization and migration of endothelial cells is regulated via the serine/threonine kinase Akt. Circ Res. 2000;86(8):892-6.

Figure legends

Figure 1.

Plasma concentrations of sVEGFR-1 (top panel), VEGF-A (middle panel) and VEGF-C (bottom panel) in RH patients before and 3 months after RDN.

Figure 2.

Plasma concentrations of NO in RH patients before and 3 months after RDN.

p indicates the difference between BSL and 3 MFU.

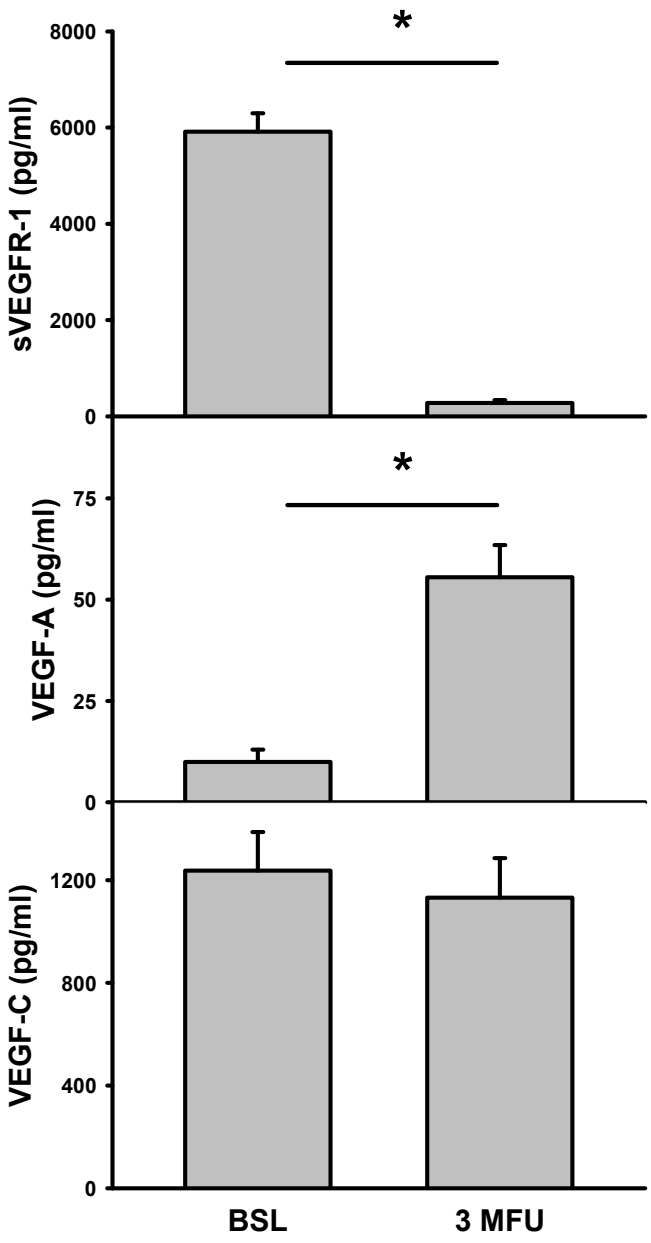
*
8000
6000
4000
2000
0
sVEGFR-1 (pg/ml)
*
75
50
25
0
VEGF-A (pg/ml)
1200
800
400
0
VEGF-C (pg/ml)
BSL
3 MFU

Figure 1.

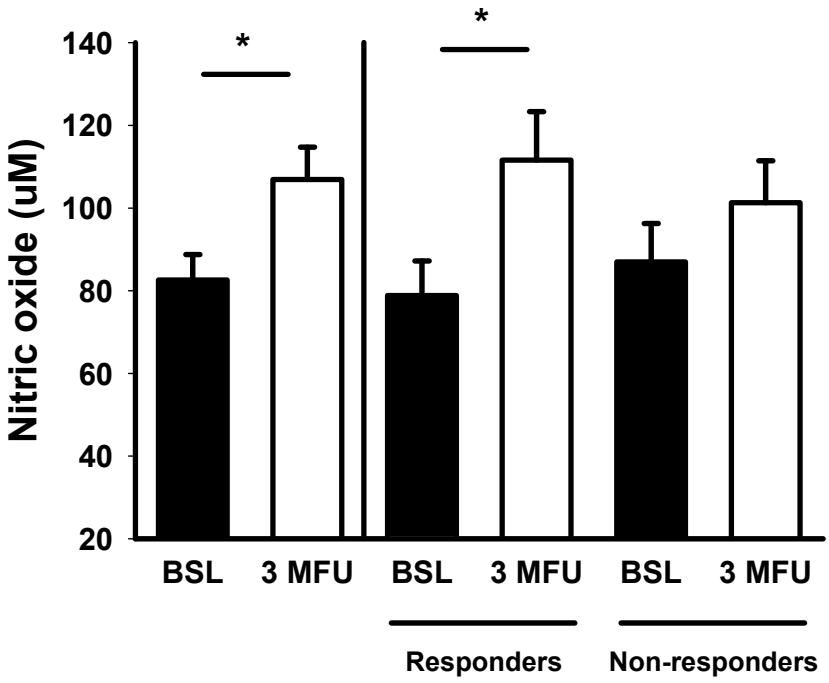
Nitric oxide (uM)
140
120
100
80
60
40
20
*
*
BSL
3 MFU
BSL
3 MFU
BSL
3 MFU
Responders
Non-responders

Figure 2.

Table 1. Baseline characteristics of study cohort

	All	Responders	Non-responders	*p value*
Age (years)	60.8±1.5	62.3±1.5	59.0±2.7	*0.682*
Gender (M/F)	37/20	23/8	14/12	
BMI (kg/m^2)	32.7±0.7	33.6±1.0	31.6±1.1	*0.212*
Office SBP (mmHg)	168.8±2.6	171.2±3.3	165.9±4.1	*0.316*
Office DBP (mmHg)	90.7±2.3	91.9±2.3	89.3±4.3	*0.755*
Heart rate (beats/min)	68.3±2.2	63.9±2.4	73.4±3.5	*0.020*
N of medications	4.6±0.2	4.7±0.3	4.5±0.4	*0.579*

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

p indicates the difference between responders and non-responders.

Table 2. The effect of renal denervation on inflammatory markers

	Responders		*p value*	Non-responders		*p value*
	BSL	3 MFU		BSL	3 MFU	
sICAM-1 (ng/ml)	206.2±8.5	216.6±8.8	*p=0.178*	212.7±8.1	226.4±13.4	*p=0.113*
sVCAM-1 (ng/ml)	691.4±25.9	748.0±27.9	*p=0.006*	678.5±30.8	726.4±38.5	*p=0.033*
sVEGFR-1 (pg/ml)	6339±502	327.7±88.7	*p<0.001*	5360±590	217.2±63.2	*p<0.001*
VEGF-A (pg/ml)	10.3±4.8	47.6±8.3	*p<0.001*	9.6±3.5	64.7±14.2	*p<0.001*

sICAM, soluble intracellular adhesion molecule 1; sVCAM, soluble vascular adhesion molecule 1; sVEGFR-1, soluble vascular endothelial growth factor receptor 1; VEGF-A, vascular endothelial growth factor A, BSL, baseline; 3 MFU, 3 months follow up.

p indicates the difference between BSL and 3 MFU.