RENAL DENERVATION REDUCES MONOCYTE ACTIVATION AND MONOCYTE-PLATELET AGGREGATE FORMATION: AN ANTI-INFLAMMATORY EFFECT RELEVANT FOR CARDIOVASCULAR RISK

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In memoriam of Prof Henry Krum

Manuscript ID: HYPE201608373D

Brief Title: Renal denervation reduces monocyte activation.

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Word Count: 5989
Abstract

Over-activation of renal sympathetic nervous system and low-grade systemic inflammation are common features of hypertension. Renal denervation reduces sympathetic activity in patients with resistant hypertension. However, its effect on systemic inflammation has not been examined. We prospectively investigated the effect of renal denervation on monocyte activation and inflammation in patients with uncontrolled hypertension scheduled for renal denervation. Ambulatory blood pressure, monocyte as well as monocyte subset activation and inflammatory markers were assessed at baseline, 3 and 6 months post-procedure in 42 patients. Renal denervation significantly lowered blood pressure at 3 months (150.5±11.2/81.0±11.2 mmHg to 144.7±11.8/77.9±11.0 mmHg), which was sustained at 6 months (144.7±13.8/78.6±11.0 mmHg). Activation status of monocytes significantly decreased at 3 months (p<0.01) and 6 months (p<0.01) post-procedure. In particular, classical monocyte activation was reduced at 6 months (p<0.05). Similarly, we observed a reduction of several inflammatory markers including monocyte-platelet aggregates (3 months p<0.01), plasma MCP-1 levels (3 months p<0.0001; 6 months p<0.05), IL-1β (3 months p<0.05; 6 months p<0.05), TNF-α (3 months p<0.01; 6 months p<0.05) and IL-12 (3 months p<0.01; 6 months p<0.05). A positive correlation was observed between muscle sympathetic nerve activity and monocyte activation before and after the procedure. These results indicate that inhibition of sympathetic activity via renal denervation is associated with a reduction of monocyte activation and other inflammatory markers in hypertensive patients. These findings point to a direct interaction between the inflammatory and sympathetic nervous system, which is of central relevance for the understanding of beneficial cardiovascular effects of renal denervation.
Keywords
Hypertension, Sympathetic Nervous System, Monocyte Activation, Inflammation, Renal Denervation.

Abbreviations
ABPM = ambulatory blood pressure monitoring
BP = blood pressure
CVD = cardiovascular disease
IL-1β = interleukin-1β
MCP-1 = monocyte chemoattractant protein-1
MPA = monocyte-platelet aggregates
MSNA = muscle sympathetic nerve activity
RDN = renal denervation
SNS = sympathetic nervous system
Introduction

Hypertension contributes significantly to the high cardiovascular morbidity and mortality rates worldwide. While acknowledging the complexity and multifactorial etiology of hypertension, it has been suggested that activation of the sympathetic nervous system (SNS) is an important contributor in at least 50% of cases (1). Persistent sympathetic activation initiates and sustains elevated blood pressure (BP) via various pathways including renal (sodium and water retention), humoral (activation of the renin angiotensin system), peripheral (vasoconstriction) and other mechanisms (1). Sustained sympathetic activation has also been closely linked to a range of clinical adverse consequences such as left ventricular hypertrophy, progression of renal disease and others (1).

Catheter-based renal denervation (RDN) is a relatively novel therapeutic approach used primarily in the context of resistant hypertension aimed at targeting sympathetic overactivity commonly encountered in these patients (2). Indeed, we and others have demonstrated that RDN reduces both renal sympathetic activity (by up to 42% in obese hypertensive dogs and by 47% in patients with resistant hypertension (3, 4)) as well as muscle sympathetic nerve activity (2). Furthermore, RDN-induced reduction in SNS observed in hypertensive patients has been associated with a significant and sustained reduction of BP at least up to 36 months (5).

Accumulating evidence suggests that cells of the innate immune system, particularly monocytes, are also implicated in the development of hypertension. Observational studies in animals and hypertensive patients have shown that elevated blood pressure is linked to pre-activated monocytes and increased levels of pro-inflammatory cytokines, which can contribute to BP elevation acutely and in the long term (6-8). In fact, increased accumulation of monocytes/macrophages has been observed in the kidneys of hypertensive animals (7).
Furthermore, genetic depletion of LysM⁺ monocytes resulted in blunted angiotensin-II induced-hypertension in mice whilst adoptive transfer of pro-inflammatory monocytes restored the BP elevation, providing direct evidence of a role for monocytes in hypertension (8).

The role of sympathetic nerves innervating the bone marrow and spleen, and the associated release of hematopoietic cells in the blood circulation has been highlighted recently (9). In cases where high activation of SNS is observed, the release of inflammatory cells into the blood circulation is amplified leading to low-grade systemic inflammation (9). These inflammatory cells have adrenergic receptors present on their cell surface (10) making them highly sensitive to catecholamine-induced modulation including cell activation. Further support for such an interaction has been derived from studies in mice with increased SNS activity triggered by exposure to chronic variable socio-environmental stressors such as cage tilting, isolation and others, in which atherosclerotic plaque formation was attenuated with blockade of the β3-adrenergic receptor on leukocytes (9, 10). These findings are indicative of relevant cross-talk between the sympathetic and the immune system, with consequences for cardiovascular regulation and cardiovascular disease (CVD) including high activation of SNS and inflammation in hypertension.

Although there is evidence showing that high SNS activation and pre-activated peripheral blood monocytes play a role in the pathogenesis of hypertension (1, 6), studies demonstrating a direct link between these two systems in patients with high BP are scarce. We therefore aimed to investigate the potential link between inflammation and SNS activity by investigating the inflammatory status of hypertensive patients before and after RDN aimed at reducing sympathetic nerve activity and BP. We examined monocyte activation using the single-chain antibody MAN-1, which specifically binds to the activated conformation of integrin MAC-1 (αMβ2; CD11b/CD18) during monocyte activation (11). The specificity of MAN-1 to detect activated monocytes has been previously shown when used in detecting
monocyte activation in patients suffering from sepsis thereby highlighting the diagnostic potential of this antibody (11). Using this unique diagnostic tool together with measurement of monocyte-platelet aggregates and other inflammatory markers we sought to investigate whether RDN-induced reduction of SNS reduces monocyte activation in hypertensive patients.
Methods

Subjects

A total of 42 consecutive patients aged 18-85 years, who were scheduled for RDN due to uncontrolled hypertension, participated in the current study. In all patients a comprehensive medical history was taken, and they underwent a thorough review of medication. In addition, biochemistry and imaging studies were obtained to exclude secondary forms of hypertension. Treating physicians and patients were instructed not to change medications during the follow-up period, except when medically required. Exclusion criteria included eGFR<15mL/min/1.73m² based on the Modification of Diet in Renal Disease criteria or a renal artery anatomy that was ineligible for RDN treatment. Based on previously published data from our laboratory (11) comparing patients with sepsis to healthy volunteers, the expected mean difference of monocyte activation measured by MAN-1 antibody binding is 12% with a standard deviation of 20%. Consequently using the paired t-test with a sigma of 0.05 and a power of 0.9, a minimal sample size of 32 was calculated. Thirty-seven patients and 39 patients out of the total 42 patients were ultimately available for the 3 and 6 month follow-up, respectively. The study was approved by The Alfred Ethics Committee in accordance with the Declaration of Helsinki and all patients provided written informed consent.

Blood pressure measurements

The 24-hour Ambulatory Blood Pressure Monitoring (ABPM) was performed using a validated device (Spacelabs 90207 or 90217 recorder; Spacelabs Healthcare, Washington, USA) in patients at baseline (n=41), 3 months (n=37) and 6 months (n=39) follow-up.
Catheter-based Radio-frequency Renal Denervation

Bilateral RDN was performed using a radiofrequency ablation catheter (Symplicity™; Medtronic Ardian Inc., Palo Alto, California, USA) as described previously (2).

Single-chain Antibody MAN-1 Production and Purification

The single-chain antibody MAN-1 was produced and purified as previously described (11, 12).

Flow Cytometry

Citrated peripheral blood was collected before RDN as well as 3 months and 6 months post-procedure. Blood samples were lysed with BD FACS Lysing Solution (1x final solution; BD Bioscience), washed and stained for 15 minutes in the dark on ice with single-chain antibody MAN-1 (10µg/ml) detected via secondary Alexa Fluor-488 anti-His-tag antibody (Qiagen), CD14-PE (Beckman Coulter), CD16-APC (BD Bioscience) and CD61-PERCP (BD Bioscience) for identification of the different monocyte cell populations. Once samples have been processed, cells were fixed immediately with BD Cell FIX™ solution (1x final solution; BD Bioscience) to prevent transitory activation of monocytes and platelets. Labeled and fixed samples were immediately analyzed or within a maximum of 24 hours analyzed by flow cytometry on a FACSCantoII (BD Bioscience). Prior to every run, BD cytometer setup and tracking beads (BD Bioscience) were used for internal calibration. Appropriate controls were prepared for each subject to allow compensation and detect non-specific binding. The cellular fluorescence was quantified as mean fluorescence intensity (MFI) or percentage of the double positive CD14⁺CD61⁺ cells at all time points. All results have been analyzed using...
BD FACS Diva software (BD Bioscience). MAN-1 binding in healthy volunteers demonstrated an inter-assay variation of less than 10% (S3).

**Imaging Monocyte-Platelet Aggregates (MPA)**

Citrated peripheral blood was lysed, washed, stained with CD14-PE and CD41-FITC (Beckman Coulter) and fixed for identification of MPAs. Samples were analyzed using AMNIS ImageStreamX MKII (EMDMillipore). All results were analyzed using IDEAS™ v6.0.

**Measurement of Plasma Markers**

Plasma was isolated from citrated whole blood by centrifugation and stored at -80°C until further analysis. Plasma concentrations of MCP-1, IL-1β, IL-12, TNF-α and IL-6 (ELISA kits, R&D) were measured by commercially available enzyme-linked immunosorbent assays, according to the manufacturer’s instructions. All measurements below the lower limit of quantitation of each assay were recorded as zero.

**Muscle Sympathetic Nerve Activity (MSNA) Assessment**

After 15 minutes of rest, multi-unit MSNA was recorded from postganglionic sympathetic nerves using microneurography (662C-3 Nerve Traffic Analysis System, Bioengineering of Iowa University, USA), as described previously (2).
Statistical Analysis

The data are presented as mean ± SEM or otherwise stated in the text. Monocyte activation, inflammatory markers and changes in 24-hour ABPM at 3 and 6 months were compared with baseline measurements using paired Student t-test. Correlations between variables were assessed with Pearson correlation coefficient. A two-sided P<0.05 was considered statistically significant. All statistical analyzes were performed using GraphPad Prism version 6.0 (GraphPad Software).
Results

Baseline Characteristics of Study Participants

The baseline characteristics and demographics of the patients are displayed in Table 1 (S1). Patients had a mean age of 65±10 years and 67% of the patient cohort were men. There were no gender-based differences observed in the study. Hypertensive patients had a mean baseline 24-hour systolic BP of 150.5±11.2 mmHg and were on an average of 4.1±2.1 antihypertensive medications including angiotensin-converting enzyme inhibitors (43%), angiotensin II receptor blocker (62%), β-blockers (55%), calcium-channel blocker (60%), α-blockers (29%), diuretics (67%), central acting sympatholytics (40%), aspirin (31%) and statins (24%). The cohort had a mean body-mass index of 31.7±4.6 kg/m^2, eGFR of 66.5±17.7 mL/min per 1.73 m^2, CRP of 2.9±2.6 mg/L and serum creatinine of 96.4±29.7 µmol/L. A proportion of patients that underwent the RDN procedure were previously diagnosed with type-2 diabetes mellitus (31%), coronary artery disease (19%) and hypercholesterolaemia (48%).

Blood pressure effects of RDN

In line with previous reports, 24-hour ABPM demonstrated a significant reduction in BP from 150.5±11.2/81.0±11.2 mmHg to 144.7±11.8/77.9±11.0 mmHg at 3 months after RDN, which was sustained at 6 months 144.7±13.8/78.6±11.0 mmHg (Figure 1).

Monocyte activation in hypertensive patients

The activation status of peripheral blood monocytes obtained from hypertensive patients was assessed using the single-chain antibody MAN-1 at baseline as well as at 3 and 6
months after RDN. Monocytes were gated based on a double sequential gating strategy of forward and side scatter light profile and CD14 expression (Figure 2A). This subsequently allowed us to determine the level of MAN-1 binding to monocytes, as a unique measure of monocyte activation (Figure 2A). Compared to baseline, MAN-1 binding to monocytes was significantly reduced at 3 months (P<0.01; Figure 2B) and 6 months (P<0.01; Figure 2B) following RDN, indicating a decrease in monocyte activation in hypertensive patients.

Furthermore, human monocytes were further categorized into three functionally and phenotypically heterogeneous subsets: classical/CD14++CD16−, intermediate/CD14++CD16+ and nonclassical/CD14+CD16++ monocytes (Figure 2C) (13). The activation status of classical monocytes based on MAN-1 binding was decreased at 6 months after RDN (P<0.05; Figure 2D), whilst no differences were observed in the activation status of intermediate and non-classical monocyte subsets (Figure 2E-F).

**Monocyte platelet aggregates in hypertensive patients**

The formation of MPAs was used as an additional measure of monocyte activation. The presence of MPAs in circulating blood of hypertensive patients was demonstrated in an imaging flow cytometer using CD14 as monocyte and CD41 as platelet marker (Figure 3A, B). Of note, hypertensive patients demonstrated higher MPA numbers when compared to a cohort of healthy volunteers (S4). MPA formation was then determined in hypertensive patients before and after RDN. Here a protocol previously used in clinical studies was applied (14-16). Monocytes were identified using the surface marker CD14 and platelets using the integrin subunit CD61, which is highly expressed on platelets, but not to a significant, in flow cytometry detectable level on monocytes (β3; Figure 3C). Following RDN, basal MPAs significantly decreased at 3 months (P<0.01; Figure 3C) after RDN, while only a numerical
but statistically non-significant reduction was observed at the 6 month time point (P=0.1387; Figure 3C). When MPA formation was assessed for overall activated monocytes, MPA reduction was observed at 3 months (P<0.01; S5A) after RDN, when compared to baseline measurement. Interestingly, when differentiated for monocyte subtypes, classical (3 months P<0.01 and 6 months P<0.05; S5B), intermediate (3 months P<0.01; S5C) and non-classical (6 months P<0.05; S5D) MPA formation was significantly reduced after RDN.

Inflammatory cytokines and chemokines in hypertensive patients

Monocyte-related cytokines and chemokines were measured. Subsequent to RDN, MCP-1 concentration was significantly reduced at 3 months (P<0.0001; Figure 4A) and at 6 months (P<0.05; Figure 4A). Plasma IL-1β levels showed a reduction at 3 months (P<0.05; Figure 4B) and 6 months (P<0.05; Figure 4B) post-RDN. TNF-α concentration was reduced at both time points (3 months P<0.01 and 6 months P<0.05; Figure 4C) when compared to baseline measurement. Similarly, IL-12 was reduced after RDN at both time points (3 months P<0.01 and 6 months P<0.05; Figure 4D). Plasma concentration of IL-6 showed a trend in reduction at 3 months (P=0.0512; Figure 4E) and 6 months (Figure 4E) after RDN.

Association of multi-unit muscle sympathetic nerve activity (MSNA) and monocyte activation

The sympathetic outflow to the periphery was determined using MSNA recordings in a subgroup of participants. There was a positive correlation observed between baseline MSNA (bursts/min) and monocyte activation (r=0.62, p<0.05; S2 Table 2). There was also a positive correlation between changes in MSNA and monocyte activation from baseline to 3 month follow-up (Δ3 month MSNA (bursts/min) vs. Δ3 month monocyte activation, r=0.63,
p<0.05; S2 Table 2) and at 6 month follow up (Δ6 month MSNA (bursts/min) vs. Δ6 month monocyte activation, r=0.88, p<0.05; S2 Table 2).
Discussion

The main findings of the current study are the following (see also Figure 5): 1) RDN substantially reduced 24-hour ABPM up to 6 months in patients with uncontrolled hypertension 2) RDN was associated with a decrease in monocyte activation, MPA formation, and a reduction in plasma levels of monocyte-related cytokines and chemokines, and 3) a positive correlation between MSNA and monocyte activation before and after RDN was evident. Overall, these findings suggest that RDN influences BP as well as the inflammatory status of monocytes in hypertensive patients and that this is, at least in part, mediated by RDN-induced reduction in SNS activity.

The marked reduction in monocyte activation in hypertensive patients observed in the present study may prevent exacerbation of systemic inflammation, which leads to beneficial effects beyond BP reduction. In line with this, a recent study in experimental hypertension in mice, RDN prevented leukocyte and T cell activation and infiltration in the kidney thereby blunting angiotensin II induced hypertension (17). As such, dampening of subsequent progression of atherosclerotic disease at an early stage of CVD may be observed.

Human monocyte subsets are known to display heterogeneous functionality according to the expression levels of lipopolysaccharide (CD14) and FcγIII (CD16) cell surface markers. Intermediate and non-classical monocytes, collectively called CD16+, are known to have a distinct characteristic compared to classical monocytes (CD16–). The CD16+ monocytes patrol the vasculature and are associated with wound healing (18, 19). Classical monocytes, on the other hand, predict increased cardiovascular risk (20). They also express high levels of MCP-1 receptor CCR2, which plays a role in atherosclerosis by abolishing atherosclerotic lesions in mice lacking this receptor (21). In our study, we observed a reduction in the activation of classical monocytes and MCP-1 plasma levels after RDN.
Taken together with previous literature, this suggest that reduction of this subset population may reduce the risk of cardiovascular events by slowing the progression of atherosclerosis. The distinct recruitment, activation and functionality of different monocyte subsets in various stages of CVD may be the reason why no changes were observed in the activation status of intermediate and non-classical monocytes. However, a detailed study on the functionality of monocyte subsets in the setting of hypertension is warranted. Of note, in another slightly older cohort of patients with more severe uncontrolled hypertension, in whom we investigated the effects of RDN on endothelial function and related biomarkers, we could not observe a significant change in MCP-1 with RDN (22). Differences in patient characteristics, treatment regimen and sensitivity of different kits used to measure MCP-1 may account for this discrepancy.

It has also been well documented that monocytes interact with activated platelets forming pro-thrombotic MPAs, thereby playing not just a role in orchestrating inflammation but also in thrombosis (23). Previous studies have shown that MPAs are elevated in diseases that are highly inflammatory and prone to thrombus formation such as myocardial infarction and stroke (24). We identified high levels of MPAs (>30%) in hypertensive patients, which is in accordance with data published by Gkaliagkousi et al. previously (23). Healthy volunteers had a significantly lower level of circulating MPAs (~3-6%; S4). The strong reduction of MPAs seen with RDN, is consistent with MPAs being a sensitive marker of monocyte activation. Notably, the reduction of MPA formation in hypertensive patients after RDN was seen in the three different monocyte subtypes as well as in activated monocytes. As MPA formation can be driven by activation of both partners, monocytes and platelets (25, 26), the important question arises whether RDN can influence the platelet activation status as well. A question that warrants to be studied in the future, particularly because of the potential
association of platelet activation with cardiovascular events and thus potential mortality/morbidity outcome benefits of RDN.

In a subset of patients who underwent RDN, MSNA was recorded in order to measure real time sympathetic nerve activity. Our results showed a positive correlation in baseline MSNA and monocyte activation. Furthermore, a reduction in MSNA activation at 3 months and 6 months after RDN also showed a positive correlation with the decrease in monocyte activation in this patient cohort, thereby further highlighting the relationship between SNS and innate immune system in human hypertension. Indeed, RDN, a therapeutic approach that targets increased sympathetic nerve activity, can dampen monocyte activation and systemic inflammation, which can potentially lead to reduced risk for cardiovascular events in hypertensive patients (Figure 5).

The slight increase in MPA and the cytokines and chemokines measured at 6 months following RDN compared to the 3 month time point raises the question of whether the reduction in inflammatory markers will be maintained in the long term. To date, there have been no reports on re-innervation of renal nerves following RDN in hypertensive patients although an animal study suggested otherwise (27). This would need to be carefully monitored in future studies with a longer patient follow-up together with application of renal noradrenaline spillover to help establish whether the effects on inflammation are reversible. Likewise, occurrence of seasonal infection and other concomitant diseases may affect monocyte activation. However, CRP levels of patients were routinely monitored and showed no changes before and after RDN (data not shown), indicating that the inflammatory status has not changed. Furthermore, while patients were specifically asked not to change any of their antihypertensive medication, we have not performed specific tests to rule this out. On the other hand, while non-adherence with some medication may well have an influence on
BP, an effect on monocyte activation, while possible, in our view would be unlikely to have had a substantial impact on the results presented.

As previously reported, RDN has been successfully used as therapy for resistant or uncontrolled hypertension. The lower baseline BP of patients enrolled in this study compared to earlier clinical trials and other studies (4, 28), may explain why the magnitude of the BP drop observed is less than what has been reported previously. While the recent sham controlled Symplicity HTN-3 trial was neutral and did not confirm a BP effect beyond that of the sham control (29), several confounding factors such medication changes, patient cohorts, and number and efficacy of ablations delivered to the renal artery question the validity of the findings (30). Of note, the experience from surgical splanchnicectomy summarized by Smithwick et al (31) indicates that while the surgery per se was associated with several side effects, it clearly decreased mortality and morbidity in hypertensive patients in spite of absence of significant reductions in BP in around half of the patients treated. Similarly, patients enrolled in other RDN trials show regression of target organ damage including reduced arterial stiffness (32) and left ventricular hypertrophy (33).

Hypertension is widely considered to represent a pro-inflammatory milieu, which may contribute to atherosclerosis and its complication frequently encountered in this patient cohort. While there is abundant experimental evidence to suggest an important role of inflammatory processes in the pathogenesis of hypertension and its cardiovascular consequences, direct evidence in humans for such an intricate interaction was scarce and less convincing yet. Furthermore, biomarkers of oxidative stress and inflammation, while frequently measured in human studies, have not yet had an impact on current risk stratification and treatment guidelines (34, 35). This may at least in part be related to the relative unspecific nature of the currently used biomarkers, which can be altered in response to a number of stimuli. Nevertheless, an imbalance in pro- and anti-inflammatory cytokines
with activation of inflammatory cells accompanied by generation of reactive oxygen species resulting in oxidative stress has been described (36, 37). Furthermore, heme-oxygenase 1 has recently been demonstrated to confer protection to the vasculature in hypertension through its modulatory role in determining the phenotype of inflammatory circulating and infiltrating monocytes (38). Also monocyte activation contributes to atherosclerosis via the generation of reactive oxygen species (37). In this context, MAN-1 binding with its unique capacity to specifically detect activation of circulating monocytes may have the potential to serve as a useful biomarker for atherosclerotic risk in patients with hypertension. Future studies will have to address this highly attractive concept in more detail.

The uncontrolled nature of the study and relatively small sample size are limitations to the current study. Inclusion of control groups, such as aged-matched and untreated subjects or a sham control group with longer term follow-up would be more scientifically robust and is required for further substantiation of our findings. However, measurements before and after RDN have provided a unique “study opportunity” to obtain proof-of-concept data that is of central relevance for the understanding of the benefits achievable by RDN.

Perspectives

Sustained activation of the SNS, an established and important factor in the pathogenesis of arterial hypertension, not only raises BP levels, but also promotes activation of circulating monocytes in hypertensive patients, providing a mechanistic link between hypertension and atherosclerotic vascular disease. Modulation of SNS activation via RDN is associated with improved BP control and also attenuates monocyte activation, thereby targeting two mechanisms relevant for cardiovascular risk. Our findings demonstrate an intricate link between the sympathetic nervous and immune system that can be targeted therapeutically and is likely to confer CV risk reduction.
Sources of Funding

This study was supported by the National Health and Medical Research Council of Australia (NHMRC), NMH was supported by a NHMRC scholarship, and GWL, MPS and KP were supported by senior fellowships from the NHMRC.

Disclosures

MPS was an investigator in studies sponsored by Medtronic. The laboratories of MPS and GWL received research funding from Medtronic, Abbott, and Servier Australia. MPS serves on scientific advisory boards for Abbott, BI, Novartis, and Medtronic and has received honoraria and travel support from Abbott, BI, Servier, Novartis, and Medtronic. GWL has received honoraria or travel support from Medtronic, Pfizer and Wyeth.
References


Novelty and significance

What is new?

- First clinical experience of beneficial effects of renal denervation on monocyte and systemic inflammation in resistant hypertension.
- Proof-of concept for an interaction between the sympathetic nervous system and the inflammatory system in human hypertension.

What is relevant?

Renal denervation-induced inhibition of sympathetic tonus not only reduces ambulatory blood pressure but also reduces monocyte activation and systemic inflammation in patients with hypertension. This finding is of central relevance for the understanding of beneficial cardiovascular effects of renal denervation.

Summary

Our results indicate that renal denervation-induced inhibition of the sympathetic nervous system activity lowers monocyte activation and inflammation in hypertensive patients. This finding represents a mechanism by which renal denervation can improve cardiovascular outcome in hypertensive patients.
Figures and Figure Legends

**Figure 1:** Changes in 24-hour ambulatory systolic and diastolic BP from baseline to 3 months and 6 months after RDN. *P<0.05, **P<0.01, ***P<0.001 compared to baseline.

**Figure 2:** (A) Monocytes were gated based on a double sequential gating of forward and side scatter light profile and CD14 expression. Then, binding of the single-chain antibody MAN-1 to these CD14+ monocytes was determined as measure of monocyte activation. (B) Activation of the overall monocyte population at baseline, 3 months and 6 months after RDN. (C) Monocyte subsets were classified according to their CD14 and CD16 cell surface expression. (D) Activation of classical monocytes, (E) intermediate monocytes and (F) non-classical monocytes assessed by the single-chain antibody MAN-1 binding at baseline, 3 month and 6 month follow-ups (Mean ± SEM). Side scatter light, SSC-A; forward scatter light, FSC-A. *P<0.05, **P<0.01 compared to baseline.

**Figure 3:** (A) Bright field image of MPA in hypertensive patients. (B) Representative fluorescence images of MPA in hypertensive patients. CD14 was used for monocyte detection, CD41 for platelet detection. (C) The percentage of monocytes with adhering platelet (MPA formation) out of the overall number of monocytes at baseline, 3 month and 6 month follow-up after RDN. CD14 was used for monocyte detection, CD61 for platelet detection. Mean ± SEM. Bars represent 10μm. **P<0.01 compared to baseline.

**Figure 4:** The plasma levels of (A) Monocyte Chemoattractant Protein-1, (B) Interleukin-1β, (C) TNF-α, (D) Interleukin-12 and (E) Interleukin-6 at baseline, 3 months and 6 months after RDN. Mean ± SEM, *P<0.05, **P<0.01, ****P<0.0001 compared to baseline.

**Figure 5:** Renal denervation, a therapeutic approach that targets increased sympathetic nerve activity, can dampen monocyte activation potentially leading to reduced risk for cardiovascular events in hypertensive patients.