Gut Dysbiosis Is Linked to Hypertension

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Abstract—Emerging evidence suggests that gut microbiota is critical in the maintenance of physiological homeostasis. This study was designed to test the hypothesis that dysbiosis in gut microbiota is associated with hypertension because genetic, environmental, and dietary factors profoundly influence both gut microbiota and blood pressure. Bacterial DNA from fecal samples of 2 rat models of hypertension and a small cohort of patients was used for bacterial genomic analysis. We observed a significant decrease in microbial richness, diversity, and evenness in the spontaneously hypertensive rat, in addition to an increased Firmicutes/Bacteroidetes ratio. These changes were accompanied by decreases in acetate- and butyrate-producing bacteria. In addition, the microbiota of a small cohort of human hypertensive patients was found to follow a similar dysbiotic pattern, as it was less rich and diverse than that of control subjects. Similar changes in gut microbiota were observed in the chronic angiotensin II infusion rat model, most notably decreased microbial richness and an increased Firmicutes/Bacteroidetes ratio. In this model, we evaluated the efficacy of oral minocycline in restoring gut microbiota. In addition to attenuating high blood pressure, minocycline was able to rebalance the dysbiotic hypertension gut microbiota by reducing the Firmicutes/Bacteroidetes ratio. These observations demonstrate that high blood pressure is associated with gut microbiota dysbiosis, both in animal and human hypertension. They suggest that dietary intervention to correct gut microbiota could be an innovative nutritional therapeutic strategy for hypertension. (*Hypertension*. 2015;65:1331-1340. DOI: 10.1161/HYPERTENSIONAHA.115.05315.) ● Online Data Supplement

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Hypertension is not only the most modifiable risk factor for cardiovascular disease and stroke but also a hallmark of obesity, diabetes mellitus, and metabolic syndrome.[1] Despite recent advances in pharmacotherapy and widespread adoption of lifestyle changes, prevalence of hypertension remains high. By some estimates, the prevalence of resistant hypertension is ≈10% to 15% among the hypertensive patient population. Treatment-resistant hypertension is characterized by high norepinephrine spillover, enhanced sympathetic outflow, and dampened parasympathetic activity, thus suggesting a neurogenic component that is essential in maintaining elevated blood pressure (BP) levels.[2,3] Increasing evidence suggests that coupled to autonomic dysfunction, treatment-resistant hypertension is also accompanied by a chronic low-grade inflammatory profile that facilitates end-organ damage and perpetuates the hypertensive state.[4,5] However, the intrinsic mechanisms that contribute to sustaining the systemic inflammatory profile found in these patients are yet to be fully understood.

In the periphery, gut microbiota plays an important role in shaping a robust systemic and intestinal immune system.[6,7] It is commonly referred to as an essential acquired organ because its composition and richness are constantly adapting to the challenges presented by the environment or by the host, such as age, diet, lifestyle modifications, and disease states.[8,9] Typically, changes in gut microbiota have been related to chronic inflammatory diseases, such as asthma, allergies, inflammatory bowel disease, and infectious diseases.[10] However, recent data suggest that it may also play a role in the development and maintenance of cardiovascular disease and metabolic disorders, such as obesity, diabetes mellitus, and metabolic syndrome.[11–14]

Adult gut microbiota is diverse; it is made up of trillions of microorganisms but mainly dominated by 4 phyla: (1) Firmicutes, (2) Bacteroidetes, (3) Actinobacteria, and (4) Proteobacteria. A delicate balance in the gut microbiota composition is key in maintaining intestinal immunity and whole-body homeostasis; any disruption of this balance could lead to

devastating pathophysiological consequences. An imbalance in gut microbiota is commonly known as dysbiosis.[9] Although characterization of a healthy microbiota is at its initial stages,[15] increasing evidence suggests that changes in the ratio of the microbe communities Firmicutes (F) and Bacteroidetes (B), known as the F/B ratio, can be potentially used as a biomarker for pathological conditions.[8,16]

Several clinical trials have been conducted evaluating the use of probiotics and its effects on BP regulation. A meta-analysis of 9 randomized trials showed a significant decrease in both systolic BP (SBP) and diastolic BP in patients who consumed a daily dose of ≥10[9] CFU of probiotics.[17] This evidence indirectly suggests that gut microbiota may play a key role in the control of BP homeostasis and that any change in microbiota composition or imbalance may potentially result in hypertension. Hence, we hypothesize that hypertensive risk factors, such as genetic predisposition and diet, induce changes in the gut microbiota that results in dysbiosis, and controlling this effect may prove as an alternative treatment for this disease. This study was designed to provide evidence for this hypothesis.

Methods

The detailed description of Materials and Methods is available in online-only Data Supplement. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee. Human studies were approved by the University of Florida Institutional Review Board.

Results

Hypertension Is Associated With Alteration in Fecal Microbiota Composition in the Spontaneously Hypertensive Rat

Fecal DNA was isolated from both Wistar Kyoto rats (WKY; mean arterial pressure: 108±2 mm Hg) and spontaneously hypertensive rats (SHRs; mean arterial pressure: 148±10 mm Hg), and the bacterial loads were represented as fecal biomass DNA per milligram of feces. A significant reduction of bacterial load was found in SHRs when compared with WKY rats (Figure 1A). Subsequently, 16S ribosomal DNA sequencing and the bioinformatics alignment comparison against the SILVA nonredundant database were performed. Significant fecal microbial alterations were found in the SHRs when compared with the WKY rats. The compositions of bacterial communities were evaluated by calculating 3 major ecological parameters, including Chao richness (an estimate of a total number of operational taxonomic units present in the given community), Pielou evenness (to show how evenly the individuals in the community are distributed over different operational taxonomic units), and Shannon diversity (the combined parameter of richness and evenness). Microbial richness, evenness, and diversity were all found to be drastically decreased in the SHR group when compared with the WKY control (Figure 1A). Furthermore, weighted UniFrac analyses were used to calculate distances between the fecal samples from the WKY rats and SHRs, and 3-dimensional (3D) scatterplots were generated by principal coordinate analysis (PCoA) to visualize whether the experimental groups in the input phylogenetic tree have significantly different microbial communities. This method
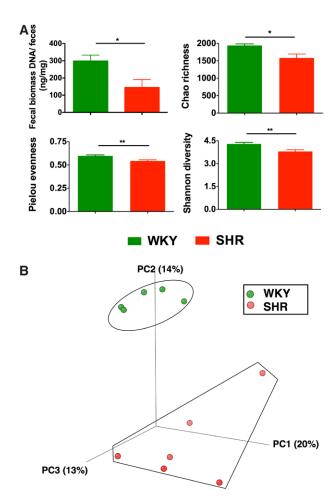

Figure 1. Gut microbial communities in Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). **A**, Decreased microecological parameters of the gut. Fecal samples were collected from WKY rats (n=5) and SHRs (n=6), and bacterial 16S ribosomal DNA were amplified and sequenced to analyze the compositions of microbial communities. Fecal biomass and microbial richness, evenness, and diversity of WKY rats and SHRs were evaluated. **B**, Principal coordinate analysis of WKY rats and SHRs. Weighted uniFrac analyses were used to calculate the distances between fecal samples from WKY rats and SHRs. Each axis percentage describes how much variation that 1-dimension accounts for. Darker colored circles indicate a point closer to the reader, whereas lighter colored circles indicate a point further away. By comparing the samples in a 3-dimensional figure, a clear separation was observed between the 2 clusters, representing WKY rats and SHRs, respectively. Results were compared by Student *t* test; *$P<0.05$ and **$P<0.01$. PC1–3 indicates principal coordinates 1–3.

allows us to present dissimilarities of the data in terms of distance.[18] Each principal coordinate axis percentage describes how much 1D can account for. The composition of the fecal microbial communities of the WKY rats and SHRs was found to be distinct, as presented in Figure 1B. A clear separation was observed in the PCoA between the 2 clusters, representing the microbial compositions of WKY rats and SHRs, indicating 2 extremely different gut environments.

An increased F/B ratio, caused by an expansion of Firmicutes or a contraction of Bacteroidetes, has been widely considered a signature of gut dysbiosis. To demonstrate whether the hypertensive rat model also confers a similar gut

microbiota pattern, we analyzed the proportions of 16S rDNA reads assigned to each phylum. The F/B ratio in the SHR was ≈5-fold higher compared with WKY rats (Figure 2A). Fecal samples from both WKY rats and SHRs were dominated by Firmicutes and Bacteroidetes and with smaller proportions of Actinobacteria and Proteobacteria (Figure 2B). However, significant differences were observed between the groups in terms of the relative abundances of Actinobacteria, Bacteroidetes, and Firmicutes. The diminished proportion of Actinobacteria indicates a less diverse microbiota in the SHR, which is consistent with the decrease in Chao richness and Shannon diversity.

Acetate and Butyrate Fermenters Decreased in the SHR

Given an imbalanced F/B ratio in the SHR, we asked what genera of bacteria contributed to the alteration of microbiota composition toward dysbiosis. LEfSe performed at the genus level demonstrated that butyrate-producing bacteria were found to be highly accumulated in WKY rats, including *Coprococcus* and *Pseudobutyrivibrio* (Figure 3A). In contrast, lactate-producing bacteria, *Streptococcus* and *Turicibacter*, were in higher quantities in the SHR. Two uncultured genera operational taxonomic units (109 and 177) that belonged to the Bacteroidetes phylum were more abundant in WKY rats. Interestingly, both of these 2 genera were completely depleted
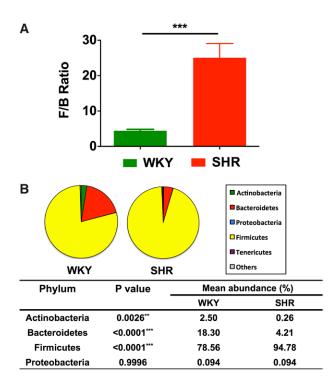
in the SHR. The abundance of other uncultured and incertae sedis bacteria genera operational taxonomic units (2761 and 3955) that belonged to Firmicutes were significantly enriched in the SHR. These changes were the major contributors to the increased F/B ratio in the SHR. *Bifidobacterium*, which belongs to the Actinobacteria phylum, is commonly considered a beneficial bacterial genus and plays a critical role in the maturation and regulation of the immune system.[19,20] The depletion of *Bifidobacterium* has been reported under multiple disease conditions[21,22] and is also a feature of dysbiosis. Remarkably, a significant depletion of *Bifidobacterium* was found in the SHR, which greatly contributed to the diminished proportion of Actinobacteria and therefore the decreased gut microbiota diversity.

To illustrate the roles of other bacteria genera in the gut, we regrouped all the bacterial 16S reads according to their major metabolic end-products as described in the Methods. Butyrate, as one of the critical bacterial metabolic products, harbors multiple beneficial properties for the host.[23,24] Some butyrate-producing bacteria can use acetate as an energy source to generate butyrate.[25] We observed ≈3-fold decrease in acetate- and a 2-fold decrease in butyrate-producing bacteria in the SHR (Figure 3B). In contrast, the abundance of lactate-producing bacteria was significantly increased in the SHR. This indicates a dysfunction in both acetogenic and butyrogenic capabilities. Thus, hypertension-associated dysbiosis is characterized as an accumulation of lactate-producing bacteria and a reduction of acetate and butyrate producers.
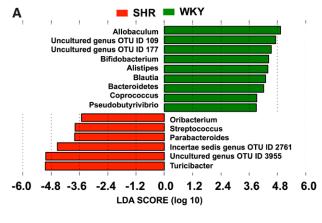
Lower Richness and Diversity of Gut Microbiota in Hypertensive Patients

On the basis of these data, we speculated that the composition of the microbiota would also differ between patients with normal (119±2 mm Hg; n=10) and high (144±9 mm Hg; n=7) SBP (Figure 4A). Fecal samples were processed and analyzed via Illumina sequencing as described for rodent experiments. We observed a reduction in bacterial Chao richness and Shannon diversity in the patients with high SBP when compared with normal SBP (Figure 4A). There was a trend toward a decrease in Pielou evenness as well, but it did not reach statistical significance with this limited number of patients.

Next, weighted UniFrac analyses were used to calculate distances, and 3D scatterplots were generated using PCoA. As shown in Figure 4B, the 2 patient populations formed separate clusters. The analyzed human data clearly confirmed the observation made in our animal model where gut dysbiosis is observed between the high and normal SBP cohorts. Nonetheless, further studies to strengthen this observation need to be conducted using a larger cohort of hypertensive patients.

Gut Microbial Diversity Is Increased by Treatment of Minocycline

Because gut microbiota can be modified through the use of broad-spectrum antibiotics, we evaluated the efficacy of using minocycline in restoring gut microbiota. Minocycline is an anti-inflammatory antibiotic that freely crosses the blood–brain barrier and has been shown to produce beneficial effects in combating hypertension.[26,27] In addition, minocycline

Phylum	P value	Mean abundance (%)	
		WKY	SHR
Actinobacteria	0.0026**	2.50	0.26
Bacteroidetes	<0.0001***	18.30	4.21
Firmicutes	<0.0001***	78.56	94.78
Proteobacteria	0.9996	0.094	0.094

Figure 2. Comparison of microbiota composition between Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). **A**, The Firmicutes/Bacteroidetes ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis. **B**, Phylum breakdown of the 4 most abundant bacterial communities in the WKY rat (n=5) and SHR (n=6) fecal samples. A decrease of Bacteroidetes along with an increase of Firmicutes resulted in a dysbiosis signature of gut microbiota in SHRs. A significant reduction of the *Actinobacteria phylum* correlated with a lower diversity value. Results were compared by Student *t* test; **P<0.01 and ***P<0.001.
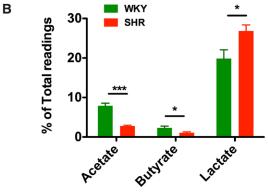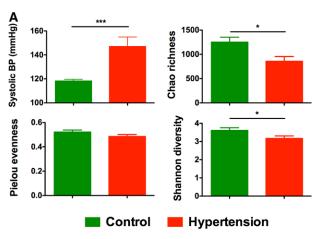
Figure 3. Diminished acetate- and butyrate-producing bacteria in spontaneously hypertensive rats (SHRs). **A**, Most enriched and depleted genera in the SHRs versus Wistar Kyoto rats (WKY). Miseq reads of bacterial 16S ribosomal DNA were analyzed and assigned to specific genera based on the sequence similarities to SILVA nonredundant 16S reference database. Linear discriminant analysis (LDA) along with effect size measurements was applied to present the enriched bacterial genera in SHRs (red) and WKY rats (green). **B**, The relative proportions of acetate-, butyrate- and lactate-producing bacteria in the gut microbiota in WKY rats (n=5) and SHRs (n=6). Sequence reads were classified according to the primary end product of the assigned bacterial genera. Genera were classified into >1 group correspondingly if they were defined as producers of multiple metabolites. Genera that were defined as producing equol, histamine, hydrogen, and propionate constituted a minor portion of the population and were therefore excluded from this analysis. Results were compared by Student *t* test; *P<0.05 and ***P<0.001. OTU indicates operational taxonomic unit.

is the drug approved in our patient studies (NCT numbers, 02133872 and 02133885), which has shown an impressive ability to decrease BP, improve hemoglobin A1c levels, and weight loss in our pilot study.[28] Therefore, understanding the effects of minocycline on microbiota will be greatly beneficial. For this purpose, we used the chronic angiotensin II (Ang II) infusion model, which is a well validated and established model of hypertension. As expected, oral minocycline for 4 weeks was able to significantly lower mean arterial pressure in Ang II–infused rats (24-h mean arterial pressure: 124±2 versus 168±2 mm Hg; Figure 5A). Consistent with the SHRs, rats infused with Ang II showed a trend toward lower bacterial load, although this finding did not reach significance (Figure 5B). Minocycline treatment did not result in a further reduction of bacterial load. This is not surprising because
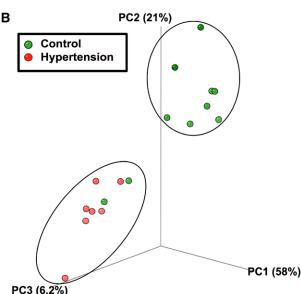

Figure 4. Reduced microbial richness and diversity in patients with hypertension. **A**, Lower microbial richness and diversity in patients with hypertension. The patients with hypertension demonstrated significantly higher office systolic blood pressure (BP) when compared with controls. Fecal samples from patients with hypertension were collected, and 16S rDNA library was prepared and analyzed by using an illumina Miseq sequencer. Richness, evenness, and diversity were used to evaluate general differences of microbial composition in different groups. **B**, Principal coordinate analysis of controls and patients with hypertension. Weighted uniFrac analyses were performed to calculate the distances between fecal samples from control and patients with hypertension. Each axis percentage describes how much variation that 1-dimension accounts for. By comparing the samples in a 3-dimensional principal coordinate analysis figure, 2 separate clusters were formed. Results were compared by Student *t* test; *P<0.05 and ***P<0.001; control: n=10 and patients with hypertension: n=7. PC1–3 indicates principal coordinates 1–3.

previous studies have indicated that the depletion of microbiota required a combination of at least of 3 antibiotics for 3 to 4 weeks,[29,30] and single antibiotics may not be sufficient to reduce the total bacterial load.[31]

To test the effect of minocycline on microbial composition, 16S rDNA sequencing was performed and data were statistically analyzed as before. Rats infused with Ang II alone demonstrated a lower richness value when compared with control (Figure 5B). Despite this lower richness value,
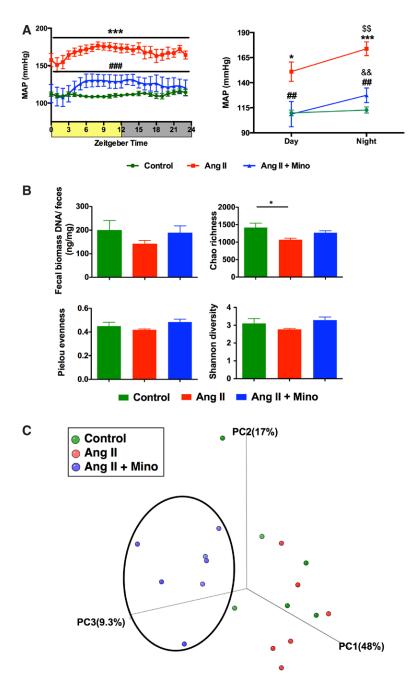
Figure 5. Minocycline (Mino) attenuates angiotensin II (Ang II) infusion hypertension and causes a shift in the gut microbiota composition. **A**, Mean arterial pressure (MAP) at 4 weeks of Ang II infusion and oral Mino treatment presented as 3-hour moving averages for 24 hours and the lowest and the highest point during day and night recording. **B**, Fecal samples were collected and bacterial 16S ribosomal DNA were amplified and sequenced to analyze the compositions of microbial communities. Fecal biomass, richness, evenness, and diversity were used to evaluate general differences of microbial composition in different groups. Ang II alone displayed a decrease in richness of gut microbiota when compared with control. **C**, Principal coordinate analysis of 3 groups. Weighted uniFrac analyses were used to calculate the distances among fecal samples from control, Ang II, and Ang II+Mino. The fecal microbiota composition shifted on minocycline treatment and differs from control and Ang II groups. Results were compared by 1-way ANOVA and Newman–Keuls post hoc test; *$P<0.05$ and ***$P<0.001$ vs control; ##$P<0.01$, ###$P<0.001$ vs Ang II; $$$$P<0.01$ vs Ang II day; &&$P<0.01$ vs Ang II+Mino day; control: n=6, Ang II: n=6, and Ang II+Mino: n=7. PC1–3 indicates principal coordinates 1–3.

we did not observe a clear separation between the clusters of these 2 groups in PCoA analysis (Figure 5C). However, Ang II+minocycline rats displayed a significant separation from the other 2 groups, indicating a shift in the gut bacterial composition by the administration of minocycline.

Most importantly, the F/B ratio was considerably increased in the Ang II–infused rats compared with controls (Figure 6A).

This was significantly reduced by minocycline treatment. In addition, the pie charts that represent the composition of microbiota at the phylum level show a trend toward relative higher abundance of Firmicutes and a lower abundance of Bacteroidetes in Ang II–infused rats when compared with controls (Figure 6B), although the differences between those 2 groups did not reach individual significance. This indicates that
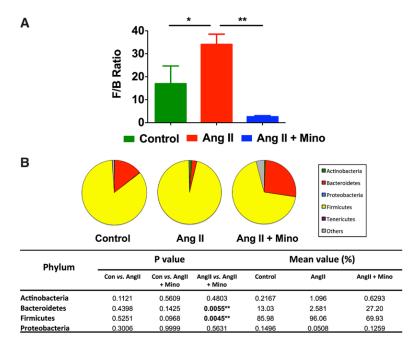
Figure 6. Comparison of microbiota composition in angiotensin II (Ang II) infusion model and minocycline (Mino) treatment. **A**, The Firmicutes/Bacteroidetes ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis. **B**, Description of mean proportional values of indicated phyla by pie chart. Gut microbiota was altered by chronic induction of hypertension (Ang II) toward dysbiosis, but Ang II+Mino administration reshaped microbiota composition toward diversity. Results were compared by 1-way ANOVA and Newman–Keuls post hoc test; *$P<0.05$ and **$P<0.01$; control: n=6, Ang II: n=6, and Ang II+Mino: n=7.

the dysbiosis in this model is characterized by both an increase in Firmicutes and a decrease in Bacteroidetes. This signature of gut microbiota dysbiosis is consistent with that previously shown in the SHR model. Interestingly, oral administration of minocycline was capable of increasing Bacteroidetes in the gut, which has been reported as a positive sign after successful fecal microbiota transplantation for the treatment of recurrent *C. difficile* infection–induced dysbiosis.[32] In addition, we observed a striking bloom of other bacteria phyla (including Verrucomicrobia) beyond Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Tenericutes, indicating a significant shift of intestinal microbial composition toward diversity. In summary, we demonstrated that minocycline administration was able to restore the Bacteroidetes population and reshape the microbiota composition, by drastically increasing microbial diversity and restoring the F/B ratio.

Acetate- and Butyrate-Producing Bacteria Are Expanded by Minocycline Treatment

Because minocycline treatment caused a remarkable shift in the gut microbial environment, we investigated the specific bacteria with notable alteration of abundance that could be verified at the genus level by LEfSe analysis and identified the 4 most expanded genera in the Ang II+minocycline–treated rats. Figure 7A shows the enrichment of *Akkermansia*, *Bacteroides*, *Enterorhabdus*, and *Marvinbryantia* in the Ang II+minocycline–treated group. Functionally, both *Bacteroides* and *Marvinbryantia* produce acetate as their main fermentation product, and *Akkermansia* and *Enterorhabdus* have been found to be inversely associated with obesity and diabetes mellitus.[33]

Similar to the SHR, the acetate- and butyrate-producing bacteria genera were also depleted in chronically infused

Ang II rats, although this finding failed to reach significance (Figure 7B). However, oral administration of minocycline resulted in a remarkable increase of both the acetate- and butyrate-producing bacteria. This was associated with a decrease in the abundance of lactate-producing bacteria.

Discussion

This study provides the first evidence, to our knowledge, of an association of hypertension with altered gut microbiota with the use of 2 different rat models of hypertension and a small cohort of hypertensive patients. The major findings of this study are (1) decreases in the microbial richness and marked increases in the F/B ratio in the animal models of hypertension, implicating the existence of gut dysbiosis in hypertension; (2) this dysbiosis was associated with decreases in acetate- and butyrate-producing bacteria and an increase in the lactate-producing bacterial population; (3) gut microbiota dysbiosis was confirmed in a small cohort of human hypertensive patients, pointing to the potential clinical significance of this work; (4) oral minocycline could rebalance the gut microbiota in a rat model of hypertension. These findings clearly implicate the role of gut microbiota in the pathophysiology of both animal and human hypertension.

Gut microbial dysbiosis has been connected to many chronic diseases, including pathophysiologies, that are associated with the cardiovascular system, such as diabetes mellitus, obesity, and cardiac dysfunctions.[12,14,16] In this study, we used 3 commonly used parameters to study the gut microbiota: Chao richness, Pielou evenness, and Shannon diversity. Although all 3 these ecological parameters were drastically decreased in the SHR model, only Chao richness was found to be lower in the Ang II infusion model of hypertension. This discrepancy
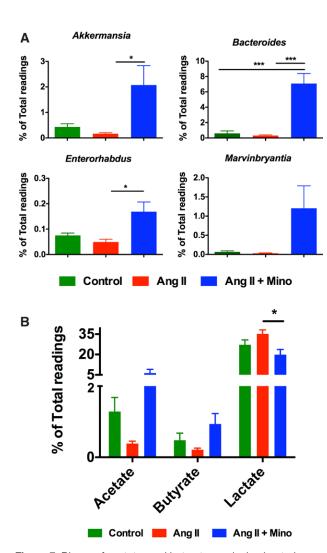
A

B

Figure 7. Bloom of acetate- and butyrate-producing bacteria on treatment of minocycline (Mino). **A,** Expansion of several genera observed in the fecal samples from rats administered with Mino and angiotensin II (Ang II). Miseq reads of bacterial 16S ribosomal DNA were analyzed and assigned to specific genera based on the sequence similarities to SILVA nonredundant 16S reference database. The most accumulated genera of bacteria were identified by LEfSe as *Akkermansia*, *Bacteroides*, *Enterorhabdus*, and *Marvinbryantia*. **B,** The relative proportions of acetate-, butyrate-, and lactate-producing bacteria in the gut microbiota in the rats either untreated or treated with Ang II or Ang II+Mino. Sequence reads were classified according to the primary end product of the assigned bacterial genera. Results were compared by 1-way ANOVA and Newman–Keuls post hoc test; *$P<0.05$ and **$P<0.01$; control: n=6, Ang II: n=6, and Ang II+Mino: n=7.

can be attributed to the differences in pathogenesis in both of these animal models and may represent an important area of future research focus. However, the most important and recognized biomarker, the F/B ratio, was dramatically increased in both the SHR and Ang II infusion models. An increased F/B ratio, caused by an expansion of Firmicutes or a contraction of Bacteroidetes, has been widely considered a signature of gut dysbiosis and has been associated with obesity, diabetes mellitus, and cardiovascular disease.[8,13,16] It is important to note that the F/B ratio is well validated in rodent and human samples; however, its significance in pig models requires further studies.[33,34] Finally, we found a reduction in the fecal

biomass in the SHR, as well as a trend toward reduction in the Ang II infusion model. This is consistent with the previous reports that dysbiosis is associated with a decrease of bacterial loads.[35] Previous studies failed to identify differences in the gut microbiota between WKY rats and SHRs,[36] and L-NAME hypertension.[37] However, the discrepancies may be accounted for by (1) significant pathophysiological differences between WKY rat and SHR colonies depending on origin, breeding, and maintenance protocols; (2) lack of details about animal housing or pathogen-free facilities; (3) using a large enough number of rats necessary to detect a differences; and (4) choosing the best models available for human hypertension.

Increases in bacterial populations relevant to the production of acetate and butyrate and reduced microbial diversity are linked to diabetes mellitus, obesity, and cardiac dysfunctions.[38] Our observations are consistent with these reports and support the important role of microbial diversity in hypertension. Of particular interest is the finding that lactate-producing bacteria were increased in both animal models of hypertension because plasma lactate levels have been associated with increased BP.[39,40] However, we did not measure plasma lactate in any of our experiments, and this remains a future direction. In addition, a greater microbial diversity of the gut is conducive to enrichment of bacterial colonies that are known to produce metabolites, such as short-chain fatty acids (SCFAs), that are beneficial in maintaining normal physiological homeostasis. Interestingly, decreases in the butyrate- and acetate-producing bacteria were found in both models of hypertension. Some butyrate-producing bacteria can use acetate as an energy source to generate butyrate.[25] Butyrate, one of the most beneficial SCFAs, harbors multiple effects for the host, such as reduction of inflammation in the intestine and adipose tissue,[41] improved insulin sensitivity in type 2 diabetes mellitus,[23,42] protection against diet induced obesity,[43] and cardiovascular disease.[44] This SCFA plays a regulatory role in the epithelial cell differentiation, barrier function, stimulation of regulatory T cells,[24] amelioration of mucosal inflammation, and suppression of colorectal cancer to name a few effects in the intestine.[45] Systematically, butyrate has been shown to prevent immune cell infiltration[46] and improve renal function following ischemic damage.[47] Another SCFA, propionate, has been shown to induce vasodilation and acute hypotensive response in wild-type mice, mediated by Gpr41 receptor.[48] Therefore, we can conclude that the hypertension-associated gut dysbiosis is characterized by an imbalance in specific microbial populations and their corresponding metabolites, specifically decreased acetate and butyrate-producing bacteria and increased lactate-producing bacteria. However, further experiments demonstrating the direct and indirect effects of SCFAs on BP are necessary.

Our previous studies have indicated that minocycline influences microglial activation and neuroinflammation in autonomic brain regions.[27] Minocycline is an anti-inflammatory antibiotic that penetrates through the blood–brain barrier and causes a reduction in high BP. Doxycycline belongs to the same antibiotic class but has limited ability to pass through the blood–brain barrier, and its effects on BP are inconclusive because there are multiple conflicting reports.[25,49] In this study, we chose to focus on minocycline as it is the focus of

our approved clinical studies, and therefore, understanding the effects of this broad-spectrum antibiotic on the gut microbiota homeostasis is crucial. Oral minocycline treatment results in the attenuation of high BP induced by chronic Ang II infusion. The first important finding was that minocycline did not reduce fecal biomass, providing evidence that this antibiotic does not have negative effects on the gut microbiota. In addition, our data show that this treatment resulted in a drastic shift in the microbial environment characterized by a bloom of diverse bacteria in the gut. A dramatic increase in Verrucomicrobia phylum bacteria was observed, indicating that minocycline produced an intestinal environment that was extremely conducive to nurturing the growth of bacteria of this phylum. Further analysis of Verrucomicrobia phylum revealed a significant increase in the mucin-degrading bacterium, *Akkermansia*. It is pertinent to point out that a decrease in *Akkermansia* has been associated with obesity and diabetes mellitus, and introduction of *Akkermansia* could reverse the metabolic disorder and improve inflammation in diabetic and obese patients.[50] In addition, preliminary data indicate that minocycline treatment was able to reverse the Ang II–induced decrease in cingulin in the colon. Cingulin is a tight junction protein whose decreased levels have been associated with gut barrier dysfunction leading to increased inflammation.[51] Thus, these gut-specific effects of minocycline seem to be linked with minocycline's beneficial effects on inflammation and hypertension.

This raises an important question, is minocycline's antihypertensive effect primarily on the gut, or on the brain or simultaneously on both, resulting in the regulation of gut–brain axis? There is no evidence to rule out either possibility at the present time because minocycline can freely cross the blood–brain barrier[26] and can directly influence both the gut and the brain. Minocycline has profound effects on the gut: (1) it inhibits expression of proinflammatory cytokines and intestinal damage in mouse model of colitis,[52,53] (2) it attenuates gut damage and intestinal mucositis because of cancer chemotherapy,[54] and (3) our data show that it improves gut microbial homeostasis during hypertension dysbiosis and is able to rebalance the gut acetate-, butyrate-, and lactate-producing bacterial populations. Furthermore, evidence of direct anti-inflammatory effects of minocycline in the brain in many neurodegenerative and neurobehavioral diseases also exists.[49,55]

We propose the following hypothesis: prohypertensive signals (eg, diet, genetic predisposition, and obesity) affect gut microbial composition, inducing dysbiosis, increase inflammatory cells in the bone marrow, and blood,[56] as well as hematopoietic stem cells. This increase in hematopoietic stem cells has been implicated in their extravasation into the brain and establishment of neuroinflammation in many neural diseases.[57,58] The combination of gut dysbiosis, peripheral inflammation, and neuroinflammation leads to the development, establishment, and maintenance of high BP. Further studies are needed to determine whether the gut dysbiosis precedes the high BP and is crucial for the development of hypertension or is rather important in the late stages of the disease. In addition, we postulate the following sequence of events in minoinduced antihypertensive effects: (1) in addition to crossing the blood–brain barrier and inhibiting microglial activation,

minocycline treatment induces an anti-inflammatory environment in the gut, which is conducive to enrichment of bacterial colonies that are known to produce metabolites, such as SCFAs, that are beneficial in maintaining normal physiological homeostasis; (2) these metabolites directly or indirectly affect the generation of hematopoietic stem cells from the bone marrow, decreasing the levels of myeloid progenitors; (3) this would result in attenuation of peripheral inflammation and decrease in mobilization of myeloid cells to the brain. The latter would be associated with attenuation of neuroinflammation. Future experiments will be aimed to provide evidence for this hypothesis.

In summary, our study demonstrates that hypertension is associated with gut microbiota dysbiosis, characterized by an increased F/B ratio, as well as a drastic decrease in acetate-, butyrate-, and an accumulation of lactate-producing microbial populations in 2 rat models of hypertension. Treatment with oral minocycline dose that attenuates hypertension also produces beneficial effects on dysbiosis. Most importantly, this study does indeed demonstrate a dysbiotic gut profile in patients with high BP. It is important to mention that the 2 control subjects observed within the hypertension PCoA plot distribution pertain to subjects who were currently under antihypertension treatment although optimum medication combination and dosage had not been met. Therefore, this suggests that reversing the gut dysbiosis might be affected by the stability of BP control and the time undergoing such treatment. This clinical trial continues to recruit patients to be able to further establish the profile of the hypertension gut dysbiosis, in addition to provide enough patients to perform subgroup analyses. Nonetheless, these observations support our overall proposal of a microbial dysbiosis linked to hypertension.

Perspectives

The involvement of gut dysbiosis in the pathogenesis of many diseases, including diabetes mellitus, obesity, cancer, and mental disorders, is rapidly emerging. However, little is known about the role of microbiota in hypertension. In this study, we present evidence of gut dysbiosis in hypertension for the first time. This includes (1) significant depletion of bacterial richness and increased F/B ratio in 2 rat models of hypertension, (2) decreases in SCFA-producing bacteria and increase in lactate-producing bacterial populations, (3) treatment with minocycline that attenuates high BP and is able to rebalance gut microbiota, and (4) validation of this concept in a small cohort of hypertensive patients. These observations suggest that innovative dietary strategies that affect gut microbiota could be developed for control and treatment of hypertension.

Disclosures

None.

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Novelty and Significance

What Is New?

- This study provides evidence that gut microbial dysbiosis is associated with hypertension. Animals with hypertension exhibited decreased gut microbial richness and diversity with associated decreases in acetate- and butyrate-producing bacteria. This dysbiosis was rebalanced by minocycline treatment. In addition, these findings were validated in the human hypertensive population.

What Is Relevant?

- Despite recent advances in pharmacotherapeutics, prevalence of hypertension remains high. We proposed that gut microbial composition could be of major importance because both diet and environmental factors have significant effect on in the development and the establishment of hypertension.

Summary

Gut microbial dysbiosis has been linked to hypertension in 2 widely used rodent models of hypertension and human hypertensive patients. Treatment with minocycline that attenuates high blood pressure rebalances the hypertension-linked dysbiosis in the rodent model.