Hypertension

ORIGINAL ARTICLE

Identification of a Gut Commensal That Compromises the Blood Pressure-Lowering Effect of Ester Angiotensin-Converting Enzyme Inhibitors

Tao Yang, Xue Mei, Ethel Tackie-Yarboi, Millicent Tambari Akere, Jun Kyoung, Blair Mell, Ji-Youn Yeo, Xi Cheng, Jasenka Zubcevic, Elaine M. Richards, Carl J. Pepine, Mohan K. Raizada, Isaac T. Schiefer, Bina Joe

BACKGROUND: Despite the availability of various classes of antihypertensive medications, a large proportion of hypertensive individuals remain resistant to treatments. The reason for what contributes to low efficacy of antihypertensive medications in these individuals is elusive. The knowledge that gut microbiota is involved in pathophysiology of hypertension and drug metabolism led us to hypothesize that gut microbiota catabolize antihypertensive medications and compromised their blood pressure (BP)-lowering effects.

METHODS AND RESULTS: To test this hypothesis, we examined the BP responses to a representative ACE (angiotensin-converting enzyme) inhibitor quinapril in spontaneously hypertensive rats (SHR) with or without antibiotics. BP-lowering effect of quinapril was more pronounced in the SHR+antibiotics, indicating that gut microbiota of SHR lowered the antihypertensive effect of quinapril. Depletion of gut microbiota in the SHR+antibiotics was associated with decreased gut microbial catabolism of quinapril as well as significant reduction in the bacterial genus *Coprococcus*. *C. comes*, an anaerobic species of *Coprococcus*, harbored esterase activity and catabolized the ester quinapril in vitro. Co-administration of quinapril with *C. comes* reduced the antihypertensive effect of quinapril in the SHR. Importantly, *C. comes* selectively reduced the antihypertensive effects of ester ramipril but not nonester lisinopril.

CONCLUSIONS: Our study revealed a previously unrecognized mechanism by which human commensal *C. comes* catabolizes ester ACE inhibitors in the gut and lowers its antihypertensive effect. **(*Hypertension*. 2022;79:1591–1601. DOI: 10.1161/HYPERTENSIONAHA.121.18711.)** • **Supplemental Material**

Key Words: antihypertensive agents ■ blood pressure ■ drug resistance ■ hypertension ■ microbiota

Gut microbiota is a well-recognized participant in the onset and progression of hypertension and targeting gut microbiota has provided a new therapeutic strategy.[1] Gut dysbiosis was initially determined in rodent hypertensive models[2–4] and hypertensive patients.[5,6] Fecal mass transfer has proven that hypertension-associated gut dysbiosis contributes to the pathogenesis of hypertension,[5,7–9] in part via modulation of gut-brain communication.[10] The importance of microbiota in the regulation of blood pressure (BP) is further highlighted by studies showing that conventionalization of germ-free rats can rescue hypotension and improve vascular contractility.[11] Modification of gut microbiota or supplementation of gut microbiota-linked metabolites has shown beneficial effects on BP control in a variety of rodent hypertensive models[12–15] and clinical trials,[16–18] providing the next frontier for control of BP in a clinical setting.

Correspondence to: Tao Yang, Department of Physiology and Pharmacology, Center for Hypertension and Precision Medicine, College of Medicine and Life Sciences, University of Toledo. Block Health Science Bldg, Rm 310, 3000 Arlington Ave, Toledo, OH 43614. Email tao.yang2@utoledo.edu or Bina Joe, Department of Physiology and Pharmacology, Center for Hypertension and Precision Medicine, College of Medicine and Life Sciences, University of Toledo. Block Health Science Bldg, Rm 237, 3000 Arlington Ave, Toledo, OH 43614, Email bina.joe@utoledo.edu

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NOVELTY AND RELEVANCE

What Is New?

Our study provided evidence for a novel concept, which uncovered a previously unrecognized role of gut microbiota in the modulation of the blood pressure-lowering effect of antihypertensive medication. Specifically, we identified *Coprococcus comes* harbors esterase activity and functions as a negative regulator for ester ACE (angiotensin-converting enzyme) inhibitor.

What Is Relevant?

Our study shed a light on novel mechanisms in the pathogenesis of resistant hypertension. Provided with the ≈20% of the hypertension population are classified as rHTN and Blacks exhibit more severe and resistant hypertension, the clinical relevance is to precisely modulate gut microbiota to increase antihypertensive medications' efficacy for resistant hypertension treatment.

Clinical/Pathophysiological Implications

Since the initial demonstration of the link between gut dysbiosis and hypertension, enumerous studies have revealed multiple mechanisms by which gut microbiota modulates the blood pressure. Our current study further proves a novel concept that gut microbiota is invovled in the resistant hypertension pathogenesis through its enzymatic roles in the catabolism of antihypertensive medications. This would serve as the foundation for further studies in the field to explore how gut microbiota contributes to the resistant hypertension.

Nonstandard Abbreviations and Acronyms

ACE	angiotensin-converting enzyme
BP	Blood pressure
EC	enzyme commission
QIIME	quantitative insights into microbial ecology mmunities by reconstruction of unobserved states
SHR	Spontaneously hypertensive rat

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A complex interaction of environment-drug-diet-microbe-host has been well recognized.[19] We recently reported a novel interaction between ACE (angiotensin-converting enzyme) inhibitor and gut microbiota in the modulation of BP in rodent hypertension.[20,21] A separate case study of a 69-year-old woman with a 44-year history of treatment-resistant hypertension reported a significant decrease in BP when administration of antibiotics (vancomycin, rifampin, ciprofloxacin) was combined with antihypertensive medication (spironolactone, valsartan–hydrochlorothiazide, verapamil).[22] This suggests an important role for gut microbiota in regulating efficacy of antihypertensive medications. Indeed, gut microbiota is capable of hydrolysis, reduction, acetylation, hydroxylation, and other modifications,[23] which can influence the metabolism of medications and xenobiotics with consequences on both efficacy and toxicity. Studies have shown that gut microbiota is involved in metabolism of amlodipine[24] and lovastatin[25] by demonstrating decreases of both after incubation with rat and human fecal lysates in vitro. However, the impact of gut microbiota on the effects of these drugs in vivo was not evaluated. With advances in sequencing technology, recent studies have identified potential bacteria that may be involved in drug metabolism, and in vitro screening studies have demonstrated that many bacterial species have biotransformative activity on a variety of medications.[26,27]

Despite these advances and the established role of gut dysbiosis in hypertension, no research to date has investigated the impact of gut microbiota on antihypertensive medication in vivo. Given the clinical relevance of a significantly sized population resistant to antihypertensive medications, our current study was designed to investigate the role of gut microbiota in the modulation of the BP-lowering effect of antihypertensive drugs. In a proof-of-concept study, we compared the BP-lowering effects of quinapril, an ester ACE inhibitor, in spontaneously hypertensive rats (SHR) with or without depletion of gut microbiota by antibiotics. ACE inhibitor is a commonly prescribed first-line antihypertensive medication.[28] Approximately, 30% to 40% of ACE inhibitor are excreted in stool.[29–31] Importantly, ester ACE inhibitor is a prodrug that is potentially hydrolyzed by gut microbiota to lower its absorption. Pharmacokinetic analysis of quinapril revealed it is predominantly excreted in feces, rather than urine.[32] The findings above made quinapril well-suited to study to probe the novel concept that gut microbiota modulates the antihypertensive effect of ACE inhibitor. Strikingly, we found that depletion of gut microbiota by antibiotics potentiated the antihypertensive effects of oral but not intravenously administered quinapril in the SHR. This suggested powerful suppression of the BP-lowering effect of quinapril by the gut microbiota. Following this novel observation, analyses of the cecal and fecal microbial composition and their catabolic capacity identified a novel role for *Coprococcus comes*, a bacterial species in the Firmicutes phylum, in hydrolyzing ester ACE inhibitor in the gut of SHR, contributing to reduced antihypertensive effects.

METHODS

The rat microbiota data and materials have been made publicly available at the NCBI SRA and can be accessed at BioProject ID PRJNA817511.

This study was conducted under protocols approved by the Institutional Animal Care and Use Committee at the University of Toledo and conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The human fecal samples were collected under the approved IRB 201400233. Baseline characteristics of the subjects were summarized in Table S1.

Statistical Analysis

All the bar graph and BP data were presented as mean±SEM. Unpaired *t* test was used when 2 groups were compared. Two-way ANOVA followed by uncorrected Fisher LSD was used in BP comparison. Pearson *r* correlation and regression were conducted using Prism GraphPad 9. *r* value 0 to 0.39 is considered weak correlation; 0.40 to 0.59 is moderate correlation; 0.6 to 1 is strong correlation. Details of statistical analysis were described in each figure legend. The bacterial abundance comparisons and Kyoto Encyclopedia of Genes and Genomes Enzyme Commission (EC) 3.1.1.1 comparisons were corrected using Deseq2 with false discovery rate. $P<0.05$ and false discovery rate $P<0.05$ were considered significant.

RESULTS

Depletion of Gut Microbiota With Antibiotics Improved the Antihypertensive Effects of Quinapril

Oral administration of quinapril produced a more pronounced reduction in BP in the SHR+antibiotics group compared with the control SHR. Decreases in mean arterial pressure, systolic BP, and diastolic BP were observed within 2.5 hours of administration (Figure 1). In contrast, IV administration of quinapril showed no significant difference in mean arterial pressure between the SHR and SHR+antibiotics groups (Figure S1). This was a clear indication that gut microbiota is capable of modifying the antihypertensive effects of quinapril in the SHR.

Since antibiotics treatment significantly depletes gut microbiota, our next objective was to determine if this greater reduction in BP in SHR+antibiotics group is due to lower catabolism of quinapril. Quinapril is a lipophilic ester prodrug that is efficiently absorbed by the intestine and hydrolyzed to form its active derivative, quinaprilat, in the liver to produce antihypertensive effects. Hydrolysis of ester quinapril to quinaprilat prematurely in the gut would render it less lipophilic and thus reduce its absorption (Figure 2A). Therefore, we measured the esterase activity in the microbiota of the cecum, a metabolically active organ.[33] We observed an esterase activity in the cecal microbiota of SHR+antibiotics (Figure 2B). Moreover, in vitro incubation of quinapril with cecal bacterial lysates demonstrated a reduction in quinapril catabolism (ΔQuinapril) in the SHR+antibiotics group (Figure 2C).

Quantification of microbial content demonstrated an ≈50% reduction in bacterial 16S copy number in the cecal content (Figure S2A and S2B) and ≈40% reduction in the fecal content (Figure S2C) in the SHR+antibiotics compared with the SHR group. Pearson *r* correlation analysis demonstrated a strong positive correlation between 16S copy number and ΔQuinapril (Figure 2D), and between esterase activity and ΔQuinapril (Figure 2E), while regression analysis demonstrated a linear regression between 16S copy number and ΔQuinapril (Figure 2D), as well as esterase activity and ΔQuinapril (Figure 2E). Taken together, these observations suggest a relationship between microbial load and microbial esterase activity in the catabolism of quinapril.

Antibiotic Treatment Altered Gut Microbial Composition

16S rRNA sequencing data were analyzed using quantitative insights into microbial ecology (QIIME2; version 2021.11),[34] SILVA database (version 132),[35] and MicrobiomAnalyst.[36] Significant cluster separation between the SHR and SHR+antibiotics groups was determined (analysis of similarities, $R=0.85$, $P=0.002$; Figure 3A). The sequencing depth of 25 000 reads per sample was demonstrated using rarefaction analyses (Figure 3B). Significant enrichment in multiple bacterial genera was observed in the SHR, such as *Lachnospiraceae*, *Ruminococcus*, *Clostridium*, *Coprococcus*, *Oscillibacter*, and *Oscillospira*, upon linear discriminant analysis effect size using genus level taxonomy (Figure 3C, Tables S2 and S3).

Positive Correlation Between *Coprococcus*, Quinapril Catabolism, and Predicted Drug Metabolism Function

Next, investigated if any bacterial groups were involved in the decreased capacity to catabolize quinapril and increased BP response to quinapril in the SHR+antibiotics. Among all identified bacterial genera enriched in the SHR (depleted by antibiotics), *Coprococcus* was picked due to its consistent depletion when data were analyzed using both QIIME1 (Data not shown) and QIIME2 (Figure 4A). Functional prediction using phylogenetic investigation of communities by reconstruction of unobserved states 2[37] indicated a significant decrease in carboxylesterase EC 3.1.1.1 abundance in the gut microbiota of the SHR+antibiotics (Figure 4B, Table S4). The abundance of EC 3.1.1.1 positively correlated with the in vitro catabolism of quinapril (ΔQuinapril) by the gut microbiota (Figure 4C). Furthermore, Pearson correlation analyses showed that *Coprococcus* 2 positively correlated with carboxylesterase EC 3.1.1.1 level and ΔQuinapril (Figure 4D), while *Coprococcus 3* positively correlated

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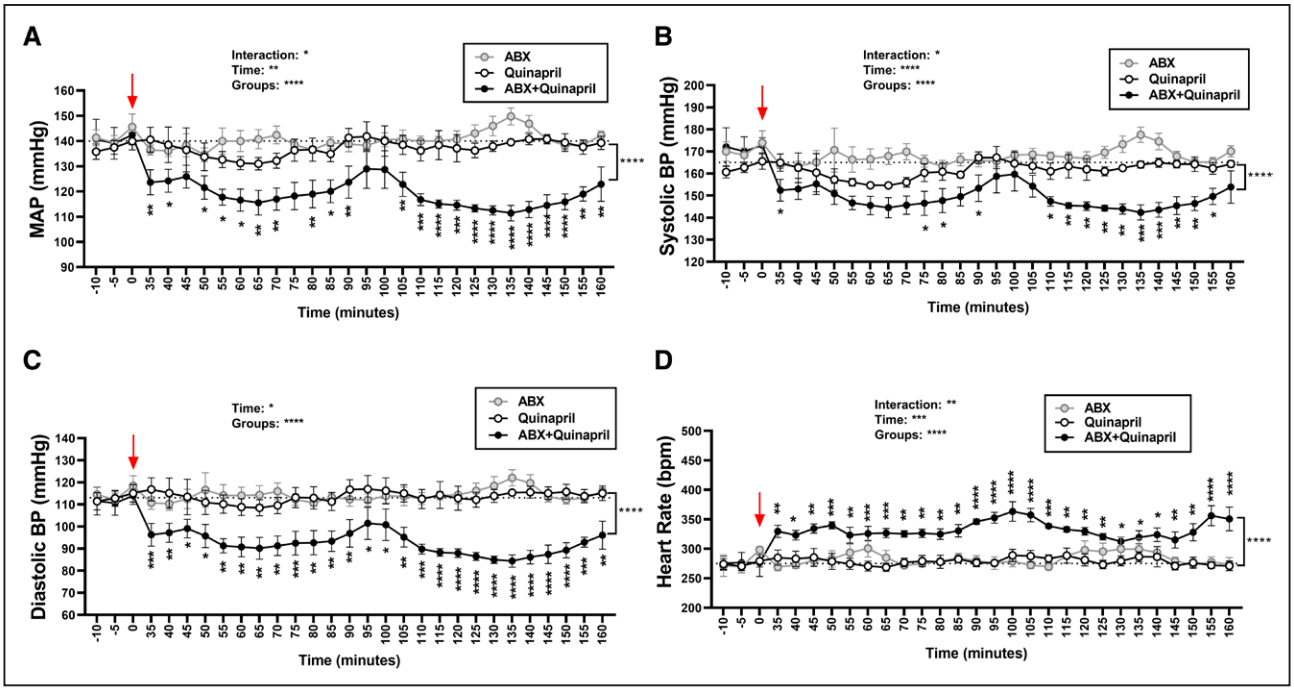

Figure 1. Increased blood pressure (BP)-lowering effects of quinapril in the male spontaneously hypertensive rat (SHR) treated with antibiotics (ABX).

Mean arterial pressure (MAP; **A**), systolic BP (**B**), and diastolic BP (**C**) were presented. A single dose of quinapril (8 mg/kg) was administered orally to SHR and SHR that were treated with a combination of vancomycin, meropenem and omeprazole. Red arrow indicates oral gavage of quinapril that was followed by 30 min recovery. BP was recorded for 10 seconds every 5 min for 3 h. ABX group N=5, quinapril group N=4, ABX + quinapril group N=4. Data were analyzed by 2-way ANOVA followed by uncorrected Fisher LSD comparison. Significant differences between quinapril group and ABX + quinapril group were noted *P<0.05; **P<0.01, ***P<0.001; ****P<0.0001.

with carboxylesterase EC 3.1.1.1 level, ΔQuinapril and cecal esterase activity (Figure 4E). Additionally, depletion of *Coprococcus* 2, *Coprococcus* 3, and carboxylesterase EC 3.1.1.1 were also detected in fecal microbiota from SHR+antibiotics group (Figure S3, Table S5 and S6).

Coprococcus comes Hydrolyzed Ester ACE Inhibitor to Reduce Their Antihypertensive Effects

To directly evaluate the catabolic activity of *Coprococcus* on quinapril, *C. comes* was cultured under anaerobic condition because of its predominant abundance in the *Coprococcus* genus. *C. comes* colonies that grew on sheep blood agar plates were round and slimy (Figure 5A). The identity of cultured colony was confirmed by 16S rRNA amplification followed by Sanger sequencing at CD Genomics. Incubation of quinapril with the bacterial lysate of *C. comes* demonstrated a potent catabolic capacity for quinapril via its esterase activity (Figure 5B and 5C). Importantly, co-administration of 6×107 CFU *C. comes* with quinapril in the SHR reduced the antihypertensive effect of quinapril in vivo (Figure 5D through 5F) compared with the SHR group that was administered with quinapril alone.

To further examine if *C. comes* catabolizes and negatively impacts other ester ACE inhibitors, we performed in vitro and in vivo experiments using ester ramipril (Figure 6A) and nonester lisinopril (Figure 6D). We demonstrated that *C. comes* was capable of catabolizing ramipril (Figure 6B) but not lisinopril (Figure 6E). Moreover, the catabolic effect of *C. comes* on ramipril resulted in a reduced BP-lowering effect of ramipril in the SHR when *C. comes* was co-administered with ramipril (Figure 6C, Figure S4A and S4B). However, the BP-lowering effect of lisinopril was not affected when co-administered with *C. comes* (Figure 6F, Figure S4C and S4D).

The Abundances of *Coprococcus* and *C. comes* in Black Hypertension and WA Hypertension in a Small Cohort of Patients

Finally, we explored the clinical relevance of the *C. comes* in ACE inhibitor disparity between Black and White American (WA) hypertensive patients. Metagenomic sequencing[38,39] of fecal samples from 16 Black and 13 WA hypertension patients indicated significant enrichment of *Coprococcus* genus and *C. comes* species in stool from Black hypertension compared with that from WA hypertension patients using uncorrected linear discriminant analysis effect size analysis (Figure S5A). Abundances of *Coprococcus* (Figure S5B) and *C. comes* (Figure S5C) are cataloged in bar graph. All the taxonomy data is included in Table S7. Since Black hypertension

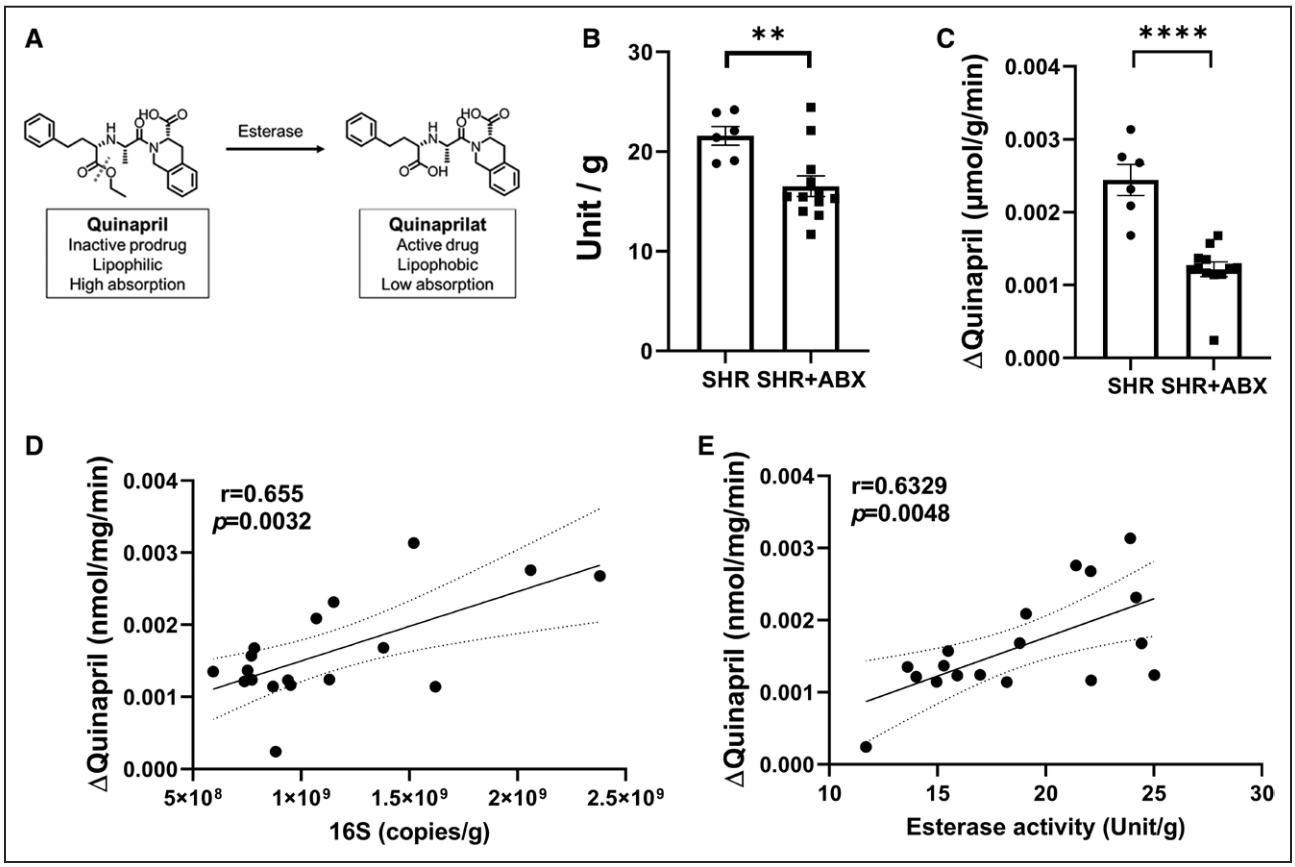

Figure 2. Decreased catabolic capacity for quinapril in the male spontaneously hypertensive rat (SHR) treated with antibiotics (ABX).

A, Quinapril is designed as a prodrug to increase its absorption in the gut and hydrolysis in the liver, thereby increasing its antihypertensive effect. We speculate that gut microbiota has esterase activity that hydrolyzes quinapril in the gut. **B**, Decreased esterase activity in the SHR+ABX. The esterase activity was measured spectrophotometrically using pNPB as a substrate. One unit of esterase activity was defined as the amount of esterase required to hydrolyze 1 μmol of pNPB to butyric acid and ethanol in one minute at pH 7.2 at 22°C. The data were presented as unit of esterase activity normalized to total protein. **C**, Less reduction of quinapril after its incubation with microbial proteins from SHR+ABX. The quantification of quinapril was determined by HPLC-MS. (D) Positive correlation between Δquinapril and 16S copy number per gram of cecal content. r=0.6841, P=0.0017. **E**, Positive correlation between Δquinapril and esterase activity. r=0.6275, P=0.0027. SHR N=6, SHR+ABX N=12. Data were analyzed by unpaired t test. **P<0.01; ****P<0.0001. Correlation was determined by Pearson r correlation and regression analysis.

patients have poor responses to ACE inhibitor,[40,41] our data suggest an interesting link of the enrichment of *C. comes* and poor ACE inhibitor response in Black hypertension, which warrants further investigations.

DISCUSSION

The current study is the first to demonstrate that (1) gut microbiota is involved in the modulation of BP-lowering effect of ACE inhibitor; (2) *Coprococcus comes* is identified to harbor esterase activity and attenuate the antihypertensive effects of ACE inhibitor; (3) specifically, the BP-lowering effect of ester ACE inhibitor, but nonester AECi is reduced by *C. comes*. Taken together, our study has uncovered gut microbiota as a previously unrecognized important modifier for the antihypertensive effect of ACE inhibitor. This serves as a foundation for assessing similar functions on other antihypertensive drugs.

Evidence for the involvement of gut microbiota in both animal models and human hypertension is unequivocal. In fact, fecal microbiota transplantation studies imply that gut microbiota alone can influence BP. Additionally, our recent studies revealed that gut microbiota may interact with captopril to modulate BP. We found that treatment of SHR with captopril, an ACE inhibitor, results in a persistent reduction in BP and gut microbial shifts in the SHR even after captopril withdrawal.[20,21] Furthermore, offspring from captopril-treated SHR mothers showed rebalanced gut microbiota, lower BP and all tested cardiovascular parameters were improved.[21] The novelty of our current study is that it shows that gut commensal *C. comes* is important for bioavailability of ester ACE inhibitor and their ability to control BP in the SHR. Evidence includes (1) depletion of gut microbiota increases antihypertensive response of oral but not IV administered quinapril. Lack

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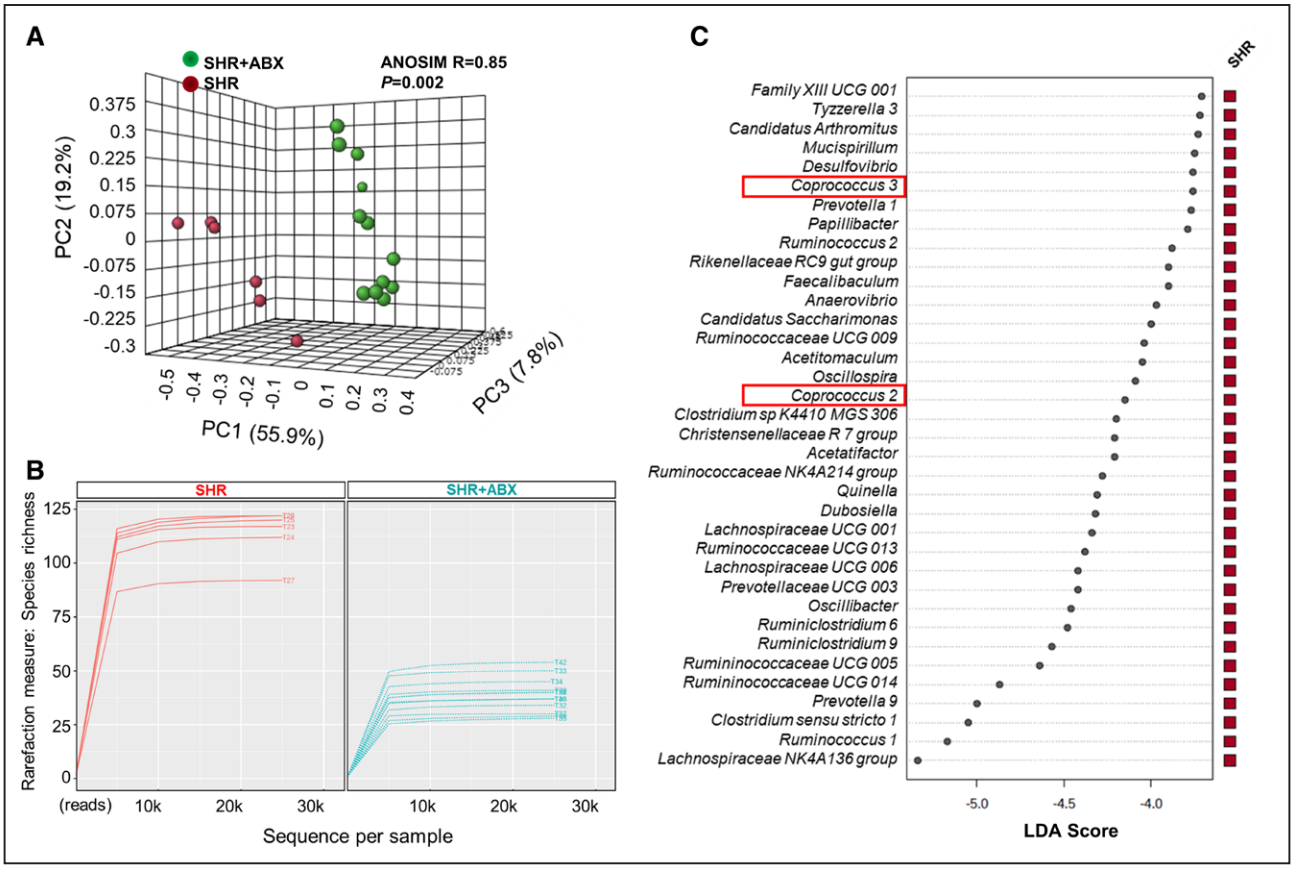

Figure 3. Alterations in the gut microbial composition in the male spontaneously hypertensive rat (SHR) treated with antibiotics (ABX).

A, Separation in the clusters of SHR and SHR+ABX was presented by Bray Curtis PCoA analysis. Analysis of similarities (ANOSIM), *R*=0.85, *P*=0.002, permutations=999. **B**, Rarefaction of species richness in the SHR and SHR+ABX, analyzed by quantitative insights into microbial ecology (QIIME2) 2021.11 against SILVA 138 database. **C**, Enrichment of bacterial genera in the SHR and SHR+ABX, analyzed by linear discriminant analysis effect size (LefSe) with corrected *P* value. SHR N=6, SHR+ABX N=12.

of decreased response to IV administration of quinapril (that bypasses the gut), reinforces the involvement of microbiota. (2) Microbiota-depleted animals showed decreased esterase activity, increased quinapril concentrations and decreased capacity of cecal microbiota to catabolize this drug. (3) Co-administration of *C. comes* with ester ACE inhibitor (ie, quinapril, ramipril) produced a reduced BP-lowering effect, compared with administration of the ACE inhibitor alone. (4) The BP-lowering effect of nonester ACE inhibitor lisinopril was not impacted in the presence of *C. comes*.

Significant alterations in the gut microbial composition were observed following antibiotics treatment. Functional prediction of the drug metabolism (other enzymes) Kyoto Encyclopedia of Genes and Genomes pathway (map00983) illustrated that carboxylesterase (EC 3.1.1.1) is one of the enzymes demonstrated in the pathway involved in the hydrolysis of irinotecan, isoniazid, and capecitabine.[42] We demonstrated positive correlations between *Coprococcus*, predicted abundance of carboxylesterase (EC 3.1.1.1) and Δquinapril. Collectively, despite the fact that prediction by phylogenetic investigation of communities by reconstruction of unobserved states 2 is less accurate than metagenomic sequencing, and Pearson correlation in non-normal data is less robust, these data raised our interest in further dissecting the microbial composition to identify bacterial taxa that may be, at least partially, responsible for the variability in BP decreases following administration of quinapril. Of all bacteria enriched in the SHR, *Coprococcus* stood out as the consistent candidate genus in our 2 rounds of analyses using both QIIME1 and QIIME2 pipelines. Notably, *Coprococcus* depletion in the SHR following antibiotics was consistent in both cecal content and fecal pellet. Indeed, our subsequent study demonstrated that *C. comes* can catabolize quinapril, and, for the first time, that co-administration of *C. comes* and quinapril significantly reduces antihypertensive effects of quinapril in vivo, strongly suggesting that microbiota composition and function need to be taken into consideration in the future design of antihypertensive medication. Importantly, we demonstrated that, the ester ACE inhibitor ramipril, but not nonester ACE inhibitor lisinopril, can also be catabolized by *C. comes*. This further expands the concept that *C. comes* harbors esterase activity to catabolize ester ACE inhibitor.

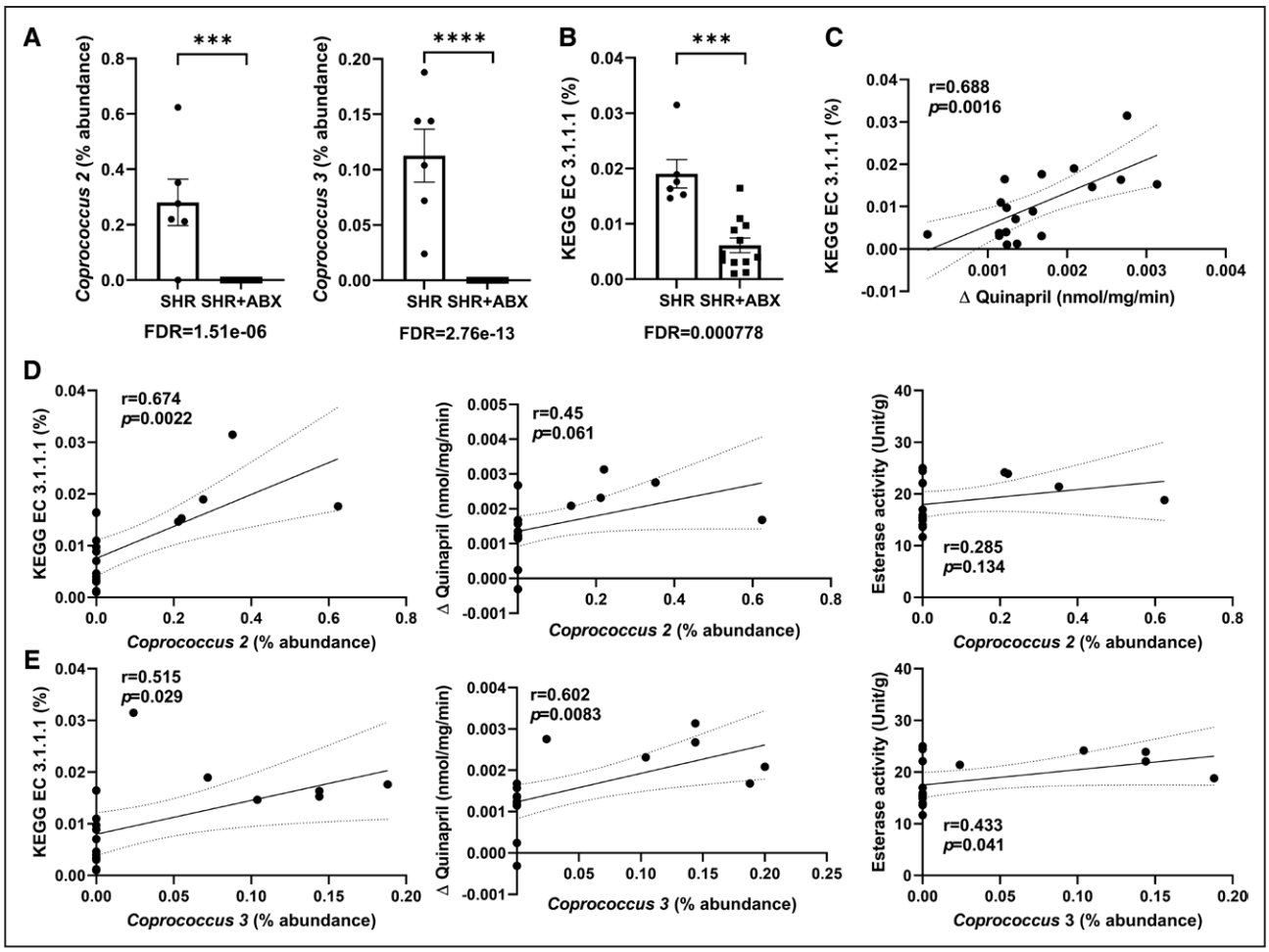

Figure 4. Positive correlations between *Coprococcus 2/3*, quinapril catabolism, and abundance of Kyoto Encyclopedia of Genes and Genomes (KEGG) enzyme commission (EC) 3.1.1.1 carboxylesterase.
Bar graphs were plotted to show the percentages of *Coprococcus* 2, *Coprococcus 3* (**A**) and KEGG EC 3.1.1.1 (**B**). **C**, Positive correlation between Δquinapril and gene abundance (%) relevant to KEGG EC 3.1.1.1 carboxylesterase. **D**, Strong positive correlation between *Coprococcus* 2 abundance and KEGG EC 3.1.1.1 (r=0.674, P=0.0022). Moderate positive correlation between *Coprococcus* 2 abundance and Δquinapril (r=0.45, P=0.061). No significant correlation between *Coprococcus* 2 abundance and esterase activity (r=0.285, P=0.134). **E**, Moderate positive correlation between *Coprococcus* 3 abundance and KEGG EC 3.1.1.1 (r=0.515, P=0.029). Strong positive correlation between *Coprococcus* 3 abundance and Δquinapril (r=0.602, P=0.0083). Moderate correlation between *Coprococcus* 3 abundance and esterase activity (r=0.433, P=0.041). P values were adjusted by DEseq2 method and denoted in **A** and **B**. ABX indicates antibiotics; and SHR, spontaneously hypertensive rat.

Finally, we analyzed and compared gut microbiota in a small cohort of Black and WA hypertension patients to determine the possible clinical significance of these animal data. As such, we did not use the STORM checklist for this study, but the rationale for our curiosity was based on 3 large trials conducted on different continents suggesting that Black hypertension respond poorly to ACE inhibitor compared with WA hypertension.[40,41,43,44] Interestingly, we found enrichment of *C. comes* in Black hypertension using uncorrected linear discriminant analysis effect size analysis, although adjusted P values did not show statistical significance in *C. comes* abundance between Black and WA hypertension patients. Nonetheless, this tempts us to postulate that increased catabolism by this species of bacteria could, in part, be responsible for poor antihypertensive response of ACE inhibitor in Black patients. However, additional experiments investigating the role of *C. comes* in the hypertension patients who are irresponsive to ACE inhibitor will be needed to support this concept.

Recent evidence from mono-colonized germ-free mice models[45] and in vitro screening assays[26] shows that a wide variety of medications and xenobiotics can be metabolized by gut bacteria. Although several studies revealed the associations between gut microbiota and drug metabolism[24,25,46]and the interactions between gut microbiota and antihypertensive drugs have been reviewed,[47–49] the hypothesis that gut microbiota interfere with the metabolism of antihypertensive medications to change their therapeutic effect in vivo, has not been tested. Our current study is the first to demonstrate the catabolic activity of a specific gut microbial species to

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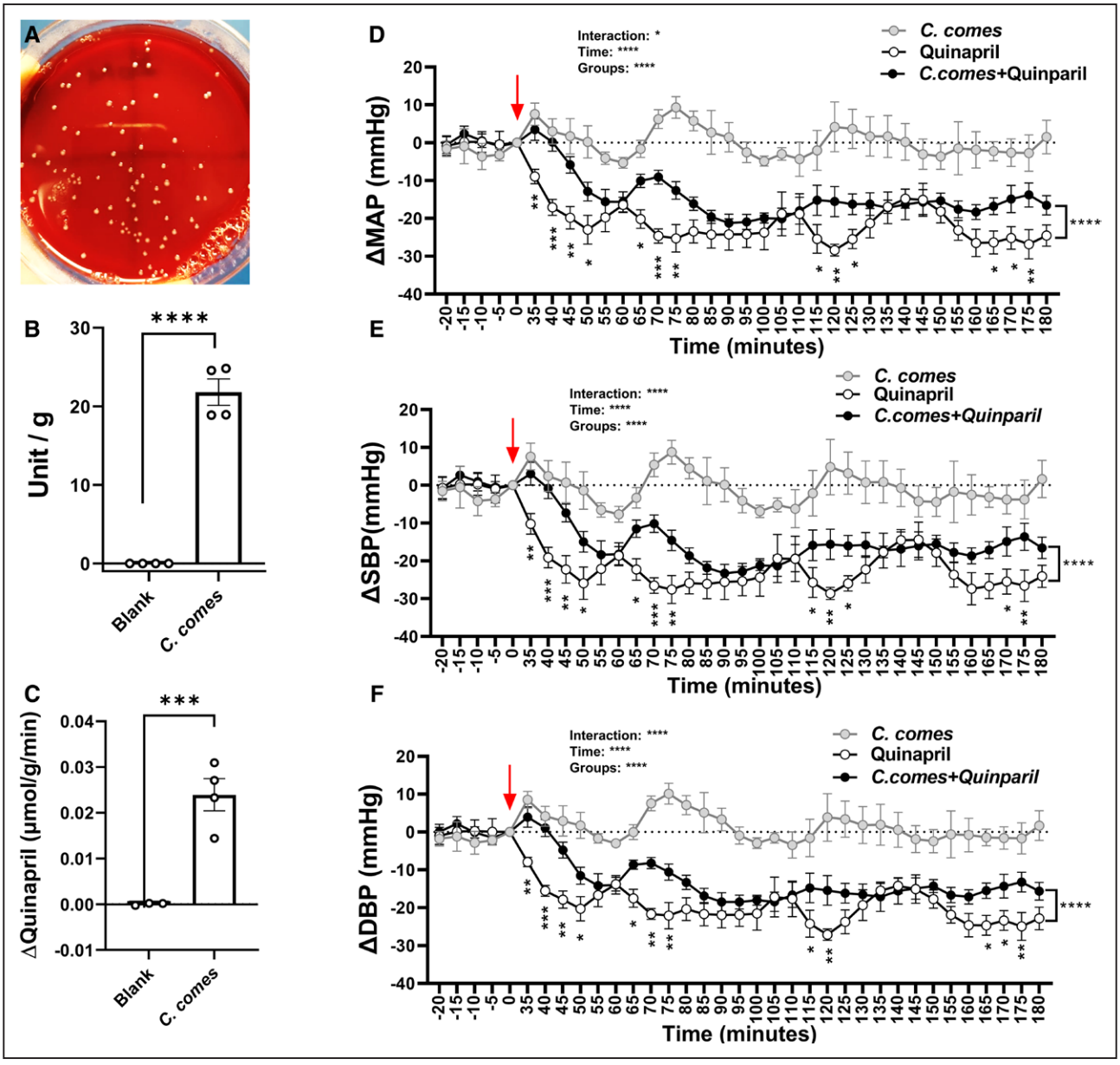

Figure 5. *Coprococcus comes* harbors catabolic capacity for quinapril and reduces its blood pressure (BP)-lowering effect. **A**, Anaerobic culture of *C. comes* on tryptic soy agar supplemented with defibrinated sheep blood. **B**, *C. comes* exhibited esterase activity, determined by spectrophotometric measurement of p-nitrophenol, a product of pNPB hydrolysis. Blank N=4, *C. comes* N=4. ****P<0.0001 in unpaired t test. **C**, Direct catabolism of quinapril by *C. comes*, measured by HPLC-MS. Blank N=3, *C. comes* N=4. ***P<0.0001 in unpaired t test. **D**–**F**, Co-administration of 6×10^7 CFU *C. comes* with quinapril resulted in a reduced BP-lowering effect of quinapril when compared with the quinapril alone group. No significant change in BP was observed in the SHR treated solely with 6×10^7 CFU *C. comes*. Red arrow indicates oral gavage of quinapril followed by 30 min recovery. BP was recorded for 10 seconds every 5 min for 3 h. *C. comes* group N=4, quinapril group N=4, *C. comes* + quinapril group N=7. Data were analyzed by 2-way ANOVA, followed by uncorrected Fisher LSD comparison. Significant differences between quinapril group and *C. comes* + quinapril group were noted *P<0.05; **P<0.01, ***P<0.001. DBP indicates diastolic BP; and SBP, systolic BP.

modulate antihypertensive effects of a widely used medication in vivo, which may have important clinical relevance for human hypertension that often presents with gut dysbiosis. We identified *C. comes* as a negative regulator of ester ACE inhibitor on BP effects in rodents in vivo. The immediate translational significance of our work is that sequencing analyses and measurement of bacterial catabolic activity in stool samples from human hypertension patients may be used as a biomarker for evaluating the antihypertensive effect of medications in the clinical setting. Additionally, manipulation of specific microbiota to enhance the bioavailability of antihypertensive medications will be of importance in future studies, whereby such microbiota could be used as probiotics. Also, other classes of antihypertensive drugs should be tested to move this hypothesis towards translation into a clinal trial.

Given that ≈20% of the hypertension population are classified as resistant hypertension and Black exhibit

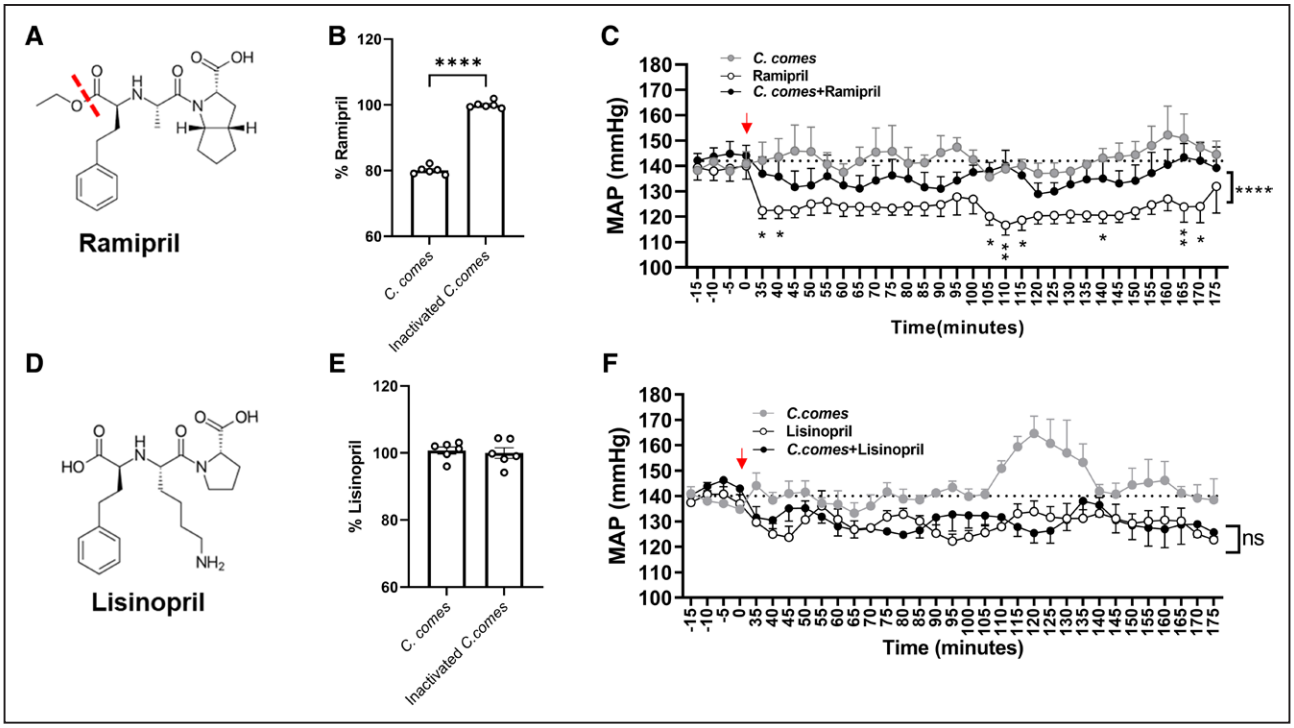

Figure 6. *Coprococcus comes* reduces the blood pressure (BP)-lowering effect of ester ramipril, but nonester lisinopril. **A**, Chemical structure of ester ramipril and targeted ester bond. **B**, 20% of ramipril was catabolized by *C. comes* in vitro. **C**, Co-administration of 6×10^7 CFU *C. comes* with ramipril resulted in a reduced BP-lowering effect of ramipril when compared with the ramipril alone group. **D**, Chemical structure of nonester lisinopril. **E**, No lisinopril was catabolized *C. comes* in vitro. **F**, Co-administration of 6×10^7 CFU *C. comes* with lisinopril did not change the BP-lowering effect of lisinopril. Ramipril and lisinopril were quantified using LC-MS/MS after their incubation with extracted proteins from *C. comes*. Heat-inactivated proteins were used as control. Data were presented as % ramipril and % lisinopril normalized to inactivated *C. comes* controls. *C. comes* group N=6, Inactivated *C. comes* group N=6. Data were analyzed by unpaired *t* test. Red arrows indicate oral gavage of respective ACE (angiotensin-converting enzyme) inhibitor followed by 30 min recovery. BP was recorded for 10 seconds every 5 min for 3 h. *C. comes* group N=3, ramipril/lisinopril group N=5, *C. comes* + ramipril/lisinopril group N=5. Data were analyzed by 2-way ANOVA, followed by uncorrected Fisher LSD comparison. Significant differences between ramipril group and *C. comes* + ramipril group at indicated time points were noted *P<0.05; **P<0.01, ***P<0.001. ****P<0.0001 between the groups ramipril and *C. comes* + ramipril. MAP indicates mean arterial pressure.

more severe and resistant hypertension,[40] this study is of great clinical significance. Recalling the clinical case report which combined the use of antibiotics and antihypertensive medications that led to a significant decrease in BP,[22] our study provides a novel concept that depletion of esterase-harboring bacteria may increase the antihypertensive effect of quinapril in rodents. Additional studies on a large-scale analysis of microbiota on ACE inhibitor resistant hypertension patients are clinically beneficial. With the progression in this promising field, precise modulation of gut bacterial strains by various antibiotics or probiotics to increase the BP-lowering effect of hypertensive drugs will push this research field to potential clinical trials to test if modification of gut microbiota can serve as a new strategy to improve the BP-lowering effect of antihypertensive drugs in hypertension and rHTN.

PERSPECTIVES

The prevalence of hypertension in adults in the United States is about 46%. Among these patients, around 20% are treatment resistant. Current treatment for resistant hypertension includes the addition or substitution of antihypertensive drugs and modulation of sympathetic activity. However, the adverse effects of overmedication, surgical invasion, and nonsustainable outcomes persist as major issues. Additionally, the pathophysiological mechanism of resistant hypertension remains elusive.

Our current study provides evidence for the concept that gut microbiota modulates the BP-lowering effect of antihypertensive medication. Particularly, we demonstrated that *Coprococcus comes* harbors esterase activity that is involved in hydrolysis of ester ACE inhibitor quinapril and ramipril. Our study revealed a previously unrecognized role of gut microbiota in the development of resistant hypertension. Identification of specific gut microbes and subsequent therapeutic targeting could provide a new avenue for the management of resistant hypertension.

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Affiliations

Department of Physiology and Pharmacology, College of Medicine and Life Sciences (T.Y., X.M., J.K., B.M., J.-Y.Y., X.C., J.Z., B.J.), UT Microbiome Consortium, Center for Hypertension and Precision Medicine (T.Y., X.M., B.M., J.-Y.Y., X.C., J.Z., B.J.), Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences (E.T.-Y., M.T.A., I.T.S.), and Center for Drug Design and Development, College of Pharmacy and Pharmaceutical Sciences (E.T.-Y., M.T.A., I.T.S.), University of Toledo, OH. Department of Physiology and Functional Genomics (E.M.R., M.K.R.) and Division of Cardiovascular Medicine (C.J.P.), University of Florida College of Medicine, Gainesville.

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Disclosures

None.

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