PERSPECTIVES IN HYPERTENSION

The gut microbiome in hypertension: The devil is in the details

RIKEISH R. MURALITHARAN

Hypertension Research Laboratory, School of Biological Sciences, Monash University, Melbourne, VIC, Australia
Victorian Heart Institute, Monash University, Clayton, Australia.

The association between the gut microbiome and hypertension is increasingly accepted. Over the past decade, our group and many others have sought to determine the role of the gut microbiome in hypertension using various approaches.[1] Our collective aim is to shift the field from association toward causation. Animal studies have shown that the gut microbiome mediates critical factors of blood pressure control, including dietary components, medication response, and immune activation.[2-4] Our team had previously shown that the protective effects of a high-fibre diet are mediated by the gut microbiome through the production of short-chain fatty acids (SCFAs) in mice. In a recent clinical study, we showed that supplementation with SCFAs reduced 24-hour systolic blood pressure in untreated hypertensive patients.[5]

Focusing on causation would allow us to target specific microbes or groups of microbes and their metabolites. This approach has been fruitful in conditions such as atherosclerosis.[6] However, current studies in hypertension do not consistently identify the same microbes. Functionally, we observe a depletion of beneficial, fibre-utilising microbes, which is not unique to hypertension. So, what are we missing? One common mistake is not considering factors that influence the gut microbiome. The gut microbiome in both humans and experimental animals is influenced by diet, living environment, sex, and genetics, amongst others, requiring careful experimental planning and adjustment. Failing to consider these intrinsic and extrinsic factors leads to confounded findings that are not replicable nor-biologically relevant findings.

In a recent study published in *Cardiovascular Research*, we investigated factors influencing the gut microbiome in the angiotensin II experimental hypertension mouse model.[7] Previous small-scale studies found that angiotensin II induced significant gut microbiome variations. Our aim was to validate these findings using a large, heterogeneous cohort of mice to quantify the extent to which angiotensin II and other experimental factors—such as diet, animal housing, sex, genotype, age, sampling site, and sequencing batch—affected gut bacterial variations.[7] We analysed 538 microbiome samples from 303 mice of different genotypes, diets, housing conditions, and age groups.[7] We confirmed that angiotensin II does induce gut microbiome variations, but it explained only 0.4% of the observed variations.[7] Factors like diet and sampling site had a more significant impact, explaining 6-6.8% of the variations.[7] We identified *Clostridium leptum* as differentially abundant between the groups.[7] Using publicly available data, we demonstrated that *Clostridium leptum* abundance was inversely associated with systolic blood pressure in both mice and hypertensive patients and positively correlated with plasma butyric acid levels (a short-chain fatty acid).[7]

This study serves as a cautionary tale, urging researchers to pay careful attention to study design, methods, and results. Moving forward, it

is essential to ensure that we consider all relevant factors to produce biologically meaningful data. Our group and others have proposed guidelines to ensure the robust design of gut microbiome studies.[8-10] We also need to accept that our understanding of the gut microbiome is still in its infancy, and as the field rapidly develops, the methods we use to study the gut microbiome will evolve accordingly.

References

1. Durgan DJ, Zubcevic J, Vijay-Kumar M, Yang T, Manandhar I, Aryal S, Muralitharan RR, Li H-B, Li Y, Abais-Battad JM, Pluznick JL, Muller DN, Marques FZ, Joe B. Prospects for leveraging the microbiota as medicine for hypertension. Hypertension. 2024;81:951-963

2. Xu C, Marques FZ. How dietary fibre, acting via the gut microbiome, lowers blood pressure. Curr Hypertens Rep. 2022;24:509-521

3. Yang T, Mei X, Tackie-Yarboi E, Akere MT, Kyoung J, Mell B, Yeo JY, Cheng X, Zubcevic J, Richards EM, Pepine CJ, Raizada MK, Schiefer IT, Joe B. Identification of a gut commensal that compromises the blood pressure-lowering effect of ester angiotensin-converting enzyme inhibitors. Hypertension. 2022;79:1591-1601

4. Saleem M, Masenga SK, Ishimwe JA, Demirci M, Ahmad T, Jamison S, Albritton CF, Mwesigwa N, Porcia Haynes A, White J, Neikirk K, Vue Z, Hinton A, Arshad S, Desta S, Kirabo A. Recent advances in understanding peripheral and gut immune cell-mediated salt-sensitive hypertension and nephropathy. Hypertension. 2024;81:436-446

5. Jama HA, Rhys-Jones D, Nakai M, Yao CK, Climie RE, Sata Y, Anderson D, Creek DJ, Head GA, Kaye DM, Mackay CR, Muir J, Marques FZ. Prebiotic intervention with hamsab in untreated essential hypertensive patients assessed in a phase ii randomized trial. Nature Cardiovascular Research. 2023;2:35-43

6. Wang Z, Roberts Adam B, Buffa Jennifer A, Levison Bruce S, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley Miranda K, DiDonato Anthony J, Fu X, Hazen Jennie E, Krajcik D, DiDonato Joseph A, Lusis Aldons J, Hazen Stanley L. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell. 2015;163:1585-1595

7. R Muralitharan R, Nakai ME, Snelson M, Zheng T, Dinakis E, Xie L, Jama H, Paterson M, Shihata W, Wassef F, Vinh A, Drummond GR, Kaye DM, Mackay CR, Marques FZ. Influence of angiotensin ii on the gut microbiome: Modest effects in comparison to experimental factors. Cardiovascular Research. 2024

8. Muralitharan RR, Snelson M, Meric G, Coughlan MT, Marques FZ. Guidelines for microbiome studies in renal physiology. American Journal of Physiology-Renal Physiology. 2023;325:F345-F362

9. Marques FZ, Jama HA, Tsyganov K, Gill PA, Rhys-Jones D, Muralitharan RR, Muir J, Holmes A, Mackay CR. Guidelines for transparency on gut microbiome studies in essential and experimental hypertension. Hypertension. 2019;74:1279-1293

10. Mirzayi C, Renson A, Furlanello C, Sansone S-A, Zohra F, Elsafoury S, Geistlinger L, Kasselman LJ, Eckenrode K, van de Wijgert J, Loughman A, Marques FZ, MacIntyre DA, Arumugam M, Azhar R, Beghini F, Bergstrom K, Bhatt A, Bisanz JE, Braun J, Bravo HC, Buck GA, Bushman F, Casero D, Clarke G, Collado MC, Cotter PD, Cryan JF, Demmer RT, Devkota S, Elinav E, Escobar JS, Fettweis J, Finn RD, Fodor AA, Forslund S, Franke A, Furlanello C, Gilbert J, Grice E, Haibe-Kains B, Handley S, Herd P, Holmes S, Jacobs JP, Karstens L, Knight R, Knights D, Koren O, Kwon DS, Langille M, Lindsay B, McGovern D, McHardy AC, McWeeney S, Mueller NT, Nezi L, Olm M, Palm N, Pasolli E, Raes J, Redinbo MR, Rühlemann M, Balfour Sartor R, Schloss PD, Schriml L, Segal E, Shardell M, Sharpton T, Smirnova E, Sokol H, Sonnenburg JL, Srinivasan S, Thingholm LB, Turnbaugh PJ, Upadhyay V, Walls RL, Wilmes P, Yamada T, Zeller G, Zhang M, Zhao N, Zhao L, Bao W, Culhane A, Devanarayan V, Dopazo J, Fan X, Fischer M, Jones W, Kusko R, Mason CE, Mercer TR, Sansone S-A, Scherer A, Shi L, Thakkar S, Tong W, Wolfinger R, Hunter C, Segata N, Huttenhower C, Dowd JB, Jones HE, Waldron L, Genomic Standards C, Massive A, Quality Control S. Reporting guidelines for human microbiome research: The storms checklist. Nature Medicine. 2021;27:1885-1892

Rikeish Muralitharan – rikeish.muralitharan@monash.edu