Molecular and Cellular Biomedical Sciences, Vol.10 No.1, March 2026, p. 1-70
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2026; 10(1): 21-31
DOI: 10.21705/mcbs.v10n1.744

Doxycycline Suppresses Hypertension through Renin–Angiotensin System (RAS) Regulation: Insights from Molecular Docking and Renal Gene Expression

Ariski Fajarido[1,2], Fadilah[3], Wawaimuli Arozal[4], Jaka Fajar Fatriansyah[5], Agrin Febrian Pradana[5], Edwina Rugayah Monayo[6]

[1]*National Brain Center Hospital Mahar Mardjono, Jakarta, Indonesia*
[2]*Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[3]*Department of Chemical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[4]*Department of Pharmacology and Theraputics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[5]*Department Metallurgical and Materials Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Indonesia*
[6]*Faculty of Medicine, Universitas Negeri Gorontalo, Gorontalo, Indonesia*

Background: Doxycycline, a tetracycline antibiotic known for inhibiting matrix metalloproteinases, has shown potential antihypertensive effects. However, its role in modulating the renin–angiotensin system remains poorly understood. This study aims to specifically evaluate Doxycycline's effects on key RAS components and blood pressure responses to clarify its underlying mechanism and support its development as a targeted antihypertensive therapeutic candidate.

Materials and methods: This study integrated an in-silico and experimental approach to assess the antihypertensive effects of doxycycline. Bioinformatics analyses were first conducted, including target prediction, gene ontology enrichment, hub-gene identification, PPI network construction, and KEGG pathway analysis, followed by molecular docking and molecular dynamics simulations to predict doxycycline's interactions with key RAS targets. To validate these computational findings, qRT-PCR was performed to measure the expression of selected genes in kidney tissues from hypertensive rats.

Results: Bioinformatics analysis identified six key target genes, including AGT, AGTR1, AGTR2, REN, ACE, and ACE2. Molecular docking showed that doxycycline exhibited stronger binding affinity to AGTR1 (-8.346 kcal/mol) than its native ligand. Molecular dynamics confirmed the stability of the doxycycline–AGTR1 complex at 20 ns. Gene expression analysis of kidney tissues from hypertensive rats revealed a significant reduction in AGTR1 expression in the group treated with doxycycline 15 mg/kg (p<0.05), while no significant change was observed at 30 mg/kg.

Conclusion: Low-dose doxycycline may modulate the renin–angiotensin pathway through AGTR1 inhibition, indicating its potential as a candidate for further antihypertensive research and warranting more comprehensive in vivo evaluation.

Keywords: *hypertension, doxycycline, molecular docking, gene expression, renin-angiotensin*

Submission: September 2, 2025
Last Revision: December 22, 2025
Accepted for Publication: December 29, 2025

Corresponding Author:
Wawaimuli Arozal
Department Pharmacology and Theraputics, Faculty of Medicine
Universitas Indonesia
Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
e-mail: wawaimuli.arozal@ui.ac.id

Copyright © 2026 Molecular and Cellular Biomedical Sciences Office.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC-BY-NC) License.

Introduction

Hypertension is closely associated with dysregulation of the renin-angiotensin system (RAS), which plays a central role in blood pressure regulation and the progression of renal injury. Pharmacological modulation of RAS components remains a cornerstone of antihypertensive therapy, however not all mechanisms governing RAS regulation have been fully elucidated. Emerging evidence suggests that doxycycline, a tetracycline antibiotic primarily known for its matrix metalloproteinase-inhibitory properties, may exert antihypertensive effects beyond its anti-inflammatory actions, although its direct involvement in RAS modulation remains unclear.[1,2] Despite the high burden in Indonesia, important aspects of its underlying pathophysiology particularly pathways involving the RAS remain insufficiently explored in translational and preclinical research.[3]

Hypertension is a chronic condition characterized by persistently elevated arterial blood pressure, commonly assessed through systolic and diastolic measurements.[4] The development of hypertension is closely associated with excessive activation of the RAS, which regulates blood pressure and vascular homeostasis. The RAS plays a central role in blood pressure regulation and vascular remodeling. Activation of the classical ACE/Ang II/AT_1R axis promotes vasoconstriction, inflammation, oxidative stress, and fibrosis, whereas the alternative ACE2/Ang-(1–7)/Mas axis exerts cardiovascular protective effects. An imbalance between these two axes has been widely reported in hypertension and cardiometabolic diseases, making the key RAS molecules (ACE, renin, AT_1R, and ACE2) rational therapeutic targets.[5–7]

The discovery of new, safe, and selective drugs remains crucials.[8] One potential candidate is doxycycline (DOX), a tetracycline antibiotic, and a broad inhibitor of matrix metalloproteinases (MMPs) is of particular interest for drug repurposing since MMPs are induced by Ang II and contribute to arterial stiffness as well as target organ damage in hypertension. Recent reviews have highlighted the involvement of MMPs in hypertension and the therapeutic potential of their inhibition (including DOX) in improving blood pressure, reducing fibrosis, and alleviating vascular dysfunction.[9]

Previous studies have provided *in vivo* evidence that DOX prevents the development of hypertension and vascular abnormalities in deoxycorticosterone acetate–salt (DOCA-salt) models by restoring gut dysbiosis and improving endothelial function. In renovascular hypertension (two-kidney, one-clip (2K1C); a RAS-dependent model), DOX suppresses MMP-2 activity, protects key contractile proteins such as sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), and limits collagen proteolysis and vascular smooth muscle cell proliferation. Although the antihypertensive effect is not always consistent, structural and functional vascular benefits have been repeatedly observed. These findings support the hypothesis that DOX may modulate the RAS axis directly or indirectly through the MMP-Ang II network.[10-12]

Although the renin–angiotensin system (RAS) is a key regulator of hypertension, integrated evidence combining molecular docking and *in vivo* RAS gene expression analysis to assess doxycycline (DOX) as an antihypertensive agent is still lacking. This knowledge gap limits understanding of the potential mechanistic role of DOX in RAS-mediated blood pressure regulation. Accordingly, this study aimed to evaluate the antihypertensive effects of DOX using hypertensive rat models supported by renal gene expression analysis. Bioinformatics-based approaches were employed to identify relevant molecular targets and pathways, followed by molecular docking to predict interactions between DOX and key RAS proteins, including ACE and AT_1R. In addition, expression analysis of major RAS components (ACE, ACE2, REN, AGTR1/AGTR2, and MAS1) was conducted to assess whether DOX alters the balance between the classical ACE/Ang II/AT_1R axis and the protective ACE2/Ang-(1–7)/Mas pathway.

Materials and methods

Study Design

The study employed an experimental laboratory design combining *in silico* analysis and *in vivo* based gene expression profiling using stored kidney tissues from hypertensive rats induced by deoxycorticosterone acetate (DOCA), salt, and uninephrectomy. All procedures performed in this study were conducted in accordance with the ethical approval as stated in the ethical statement subsection.

Animal Treatment

This study utilized stored biological samples consisting of kidney tissues from three groups of rats (n=5 per group). Hypertension was induced using a DOCA-salt model. In the DOCA group (control group), rats underwent uninephrectomy

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

followed by subcutaneous administration of DOCA at a dose of 20 mg/kg twice per week, and received 1% NaCl in their drinking water daily for five weeks to establish chronic hypertension. The DOXY 15 group received doxycycline at 15 mg/kg orally via gavage once daily for five weeks in addition to undergoing uninephrectomy, DOCA induction (20 mg/kg, subcutaneous, twice weekly), and daily 1% NaCl administration. Similarly, the DOXY 30 group received doxycycline at 30 mg/kg orally once daily for five weeks, along with uninephrectomy, DOCA induction, and daily 1% NaCl exposure.

Blood Pressure Measurement

Blood pressure was measured weekly using a fully automated CODA tail-cuff system (Kent Scientific, Torrington, USA). Rat tails were warmed to 37°C to optimize blood flow before placement of the occlusion and volume-pressure recording cuffs. Systolic and diastolic pressures were automatically recorded, and the mean of ten readings was used for analysis. All measurements were performed between 08:30 and 12:30, consistent with procedures described in previous studies.[13]

Data Collection and Processing

Predictive target genes related to hypertension were identified through two publicly available databases: the Comparative Toxicogenomics Database (CTD; https://ctdbase.org/) and the Gene Expression Omnibus (GEO) database, specifically the GSE37460 dataset (https://www.ncbi.nlm.nih.gov/geo/).[14-17] Data selection was based on keywords and biological annotations relevant to the pathophysiology of hypertension. GSE37460 was chosen because it contains gene expression profiles from tissues involved in blood pressure regulation and has been widely utilized in hypertension-related studies. The analysis results from both sources were then used to generate a list of target genes. The analysis results were used to obtain the target genes. Subsequently, Draw Venn Diagram was employed to determine the intersection between predictive target genes derived from CTD and GEO, resulting in the identification of 47 overlapping genes.[18]

Protein-Protein Interaction (PPI) Analysis

The protein–protein interaction (PPI) network was constructed using the STRING database (https://string-db.org/) with a confidence score threshold of 0.900.[19] The data were visualized in Cytoscape v3.8 with the cytoHubba plugin.[20] The top 10 hub genes were identified using the maximal clique centrality (MCC) method, which provides higher accuracy in predicting essential proteins.[21]

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis

Enrichr (https://maayanlab.cloud/Enrichr/) was employed to perform gene ontology (GO) analysis, including biological processes, molecular functions, and cellular components, as well as KEGG pathway enrichment.[22,23] A significance threshold of $p < 0.05$ was applied. KEGG enrichment results were visualized using ShinyGO 0.81 (http://bioinformatics.sdstate.edu/go/). An FDR-adjusted p-value of <0.05 was considered statistically significant.[24]

Molecular Docking Analysis

Protein structures for AGT (PDB ID: 6RV0), AGTR1 (PDB ID: 4ZUD), AGTR2 (PDB ID: 5UNH), REN (PDB ID: 2V0Z), ACE (PDB ID: 6WF2), and ACE2 (PDB ID: 1R4L) were obtained from the Protein Data Bank (PDB: https://www.rcsb.org/).[25] The structure of the doxycycline ligand was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/).[26] Molecular docking simulations were performed using Maestro Schrödinger. Ligand preparation was carried out with the following parameters: target pH 7.0±2.0, desalt, and tautomer generation, selecting the ligand with the lowest ionization penalty. Protein preparation included assigning bond orders, adding hydrogen atoms, filling in missing chains and loops using Prime, and removing water molecules beyond 5.00 Å with the system set at pH 7.0 ± 2.0. Docking was initiated by defining the grid area using the fit auto ligand option, followed by active site selection via grid receptor generation. The docking parameters applied were a van der waals scaling factor of 1.00 and a partial charge cutoff of 0.25.[27] Additionally, the native ligand of each respective protein structure was used as a positive control to validate the docking protocol.

Molecular Dynamic Analysis

Molecular dynamics (MD) simulations were conducted using Maestro Schrödinger (Schrödinger Inc). System preparation was performed with the System Builder module, including solvation minimization and the addition of ions (9 Cl^-, Na^+/Cl^- salt at 0.15 M). The fully solvated system was equilibrated and simulated under the NPT ensemble (constant number of particles, pressure, and temperature) with parameters set to 310 K, 1.01325 bar, a total simulation

time of 50 ns, a trajectory recording interval of 9.6 ps, and energy output every 1.2 ps. Prior to simulation, the system was relaxed to ensure stability. Output parameters included root mean square deviation (RMSD) and root mean square fluctuation (RMSF).[27]

Gene Expression Analysis

Total RNA was extracted from 100 mg of kidney tissue using the Direct Zol RNA MiniPrep Plus kit with TRI Reagent (Zymo Research, California, USA), and RNA purity (1.8-2.0) was verified using a NanoDrop spectrophotometer at 260 nm. cDNA was synthesized from 100 ng of total RNA using the HiScript III dRT SuperMix (Cat No. R323-01, Vazyme Biotech, Nanjing, China) protocol and qRT-PCR was performed using Taq Pro Universal SYBR qPCR Master Mix (Cat No. Q712-02, Vazyme Biotech, Nanjing, China). Actin beta (ACTB) served as the housekeeping gene for normalization. Each qRT-PCR reaction was conducted with two technical replicates, and samples represented the biological replicates of each experimental group. Thermocycling conditions consisted of denaturation at 95°C for 10 s, annealing at primer-specific t emperatures for 30 s, and elongation at 72°C for 30 s over 40 cycles. Relative gene expression was calculated using the Livak method (2^-ΔΔCt), where ΔCt values were obtained between target and reference genes and normalized to the non-hypertensive control group. Fold changes in mRNA expression were then determined to evaluate differences in target gene expression among samples.[28,29]

Statistical Analysis

The results were presented as relative gene expression values to allow clear comparison between experimental groups. All measurements were performed in duplicate to improve data reliability, and the resulting data were subjected to statistical analysis. Normality was assessed using the Shapiro–Wilk test, normally distributed data were analyzed by ANOVA followed by Tukey's HSD test with a significance threshold of $p \leq 0.05$, whereas data that non-normally distributed were evaluated using the Kruskal–Wallis test to indentify overall differences among the study groups, followed by the Mann–Whitney test for pairwise group comparisons at the same confidence level. All statistical analyses were performed using IBM SPSS Statistics version 29.0.0.0.

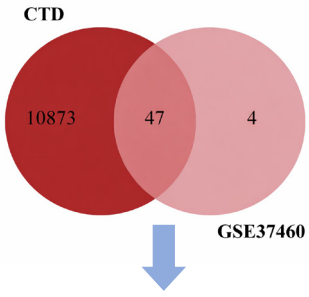
Results

Data Collection and Prossesing

Predictive target genes associated with hypertension were identified using two online databases, yielding 10924 potential target genes. The Comparative Toxicogenomics Database (CTD) contributed 10920 genes, while the GSE37460 dataset from the Gene Expression Omnibus (GEO) provided 51 genes. A Venn diagram was constructed to determine the overlapping targets among these hypertension-related genes. Cross-referencing of the datasets revealed 47 overlapping genes, which were identified as potential targets (Figure 1).

Protein-Protein Interaction (PPI)

A total of 47 target genes were analyzed for protein–protein interactions (PPI) using STRING-DB, followed by k-means clustering. The analysis identified a cluster containing 12 genes with strong interactions, primarily involved in blood volume regulation through the renin–angiotensin pathway. The top 10 hub genes based on MCC degree values were AGT, AGTR1, AGTR2, ACE, ACE2, REN, EDNRA, ALB, C3, and PLG (Figure 2). These associations indicate that the proteins function cooperatively to contribute to shared biological processes, although this does not necessarily imply direct physical binding among them. Based on their



AACS, DBH, PCCB, MAS1, IL10, PLG, ALB, EHHADH, GSTA1, HK1, ACTN2, ACSS1, FAH, DLAT, GOT1, C3, EDNRA, PFKFB1, TNF, ENO2, GGT1, AKR1B1, COMT, AGTR1, ICAM1, MET, DPYD, AGT, ACADM, ACTN1, ACE, SDC1, ALOX12B, CYP3A4, EGF, ACE2, CD14, F2R, FBP1, COL4AI, REN, AGTR2, HGF, VWF, FMO1, CCL2, KDR.

Figure 1. The prediction of potential hypertensive nephropathy genes. The diagram illustrates the overlap between the predicted target genes of hypertensive nephropathy, yielding 47 overlapping genes as potential targets.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

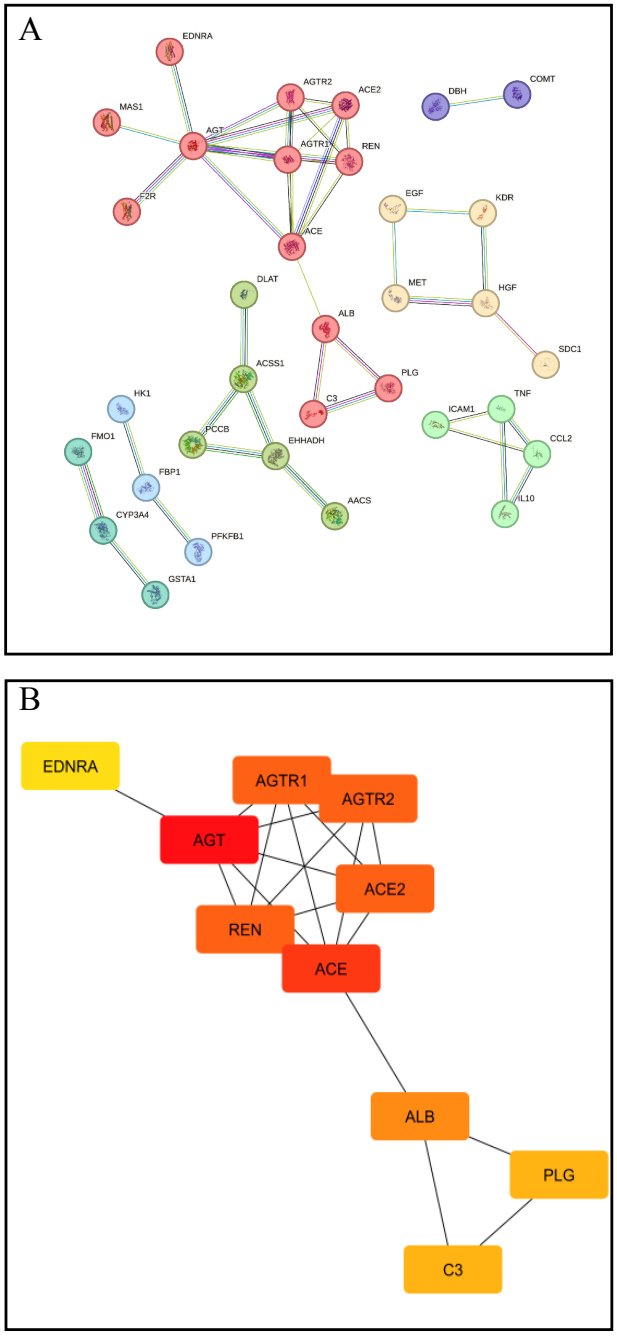


Figure 2. Results of protein-protein interaction analysis. A: Protein-protein interaction of 34 protein nodes. B: Top 10 key genes associated with hypertension.

established functions, these hub genes are recognized to play critical roles in human biology.

Gene Ontology (GO) and KEGG Pathway Analysis

Gene Ontology (GO) analysis categorized the 10 potential hub genes into three groups: biological processes, cellular components, and molecular functions. The key pathways identified included regulation of systemic arterial blood pressure by the renin–angiotensin system, blood vessel diameter maintenance, and regulation of vasoconstriction (Table 1). KEGG pathway analysis further revealed that these genes are involved in multiple hypertension-related pathways (Figure 3). Specifically, the GO results demonstrated that the target genes regulate biological processes through mechanisms associated with the renin–angiotensin pathway. These genes were also localized to the secretory granule lumen and were associated with endopeptidase activity in molecular functions. Collectively, these findings suggest that doxycycline (DOX) may exert antihypertensive effects by modulating key pathways and cellular processes.

Functional enrichment analysis was performed to identify signaling pathways related to the expressed genes. Pathways associated with hypertensive nephropathy were determined using KEGG enrichment with the Enrichr database. The top 10 enriched KEGG pathways were obtained (Figure 3), all of which were related to hypertensive nephropathy, with p-values <0.01 considered significant. The renin-angiotensin system was the most significant pathway with a p-value of 2.378×10^{-16}, and visualization with ShinyGO 0.81 revealed six expressed genes in this pathway: AGT, AGTR1, AGTR2, ACE, REN, and ACE2. Based on the enrichment results, the renin-angiotensin system (RAS) pathway was selected with a -log10 (FDR) value of 10, indicating strong significance in hypertensive nephropathy, and all identified genes were further subjected to molecular docking with doxycycline.

Molecular Docking Analysis

Molecular docking simulations demonstrated that doxycycline (DOXY) effectively interacts with the AGTR1 target protein, exhibiting a higher binding affinity than the native ligand, which suggests strong and stable ligand–receptor interactions (Table 2). DOXY engages the same 11 amino acid residues as the native ligand Arg167, Ser105, Val108, Tyr35, Trp84, Ile31, Tyr87, Thr88, Ile288, Tyr92, and Pro285 thereby enhancing binding stability (Figure 4). Based on amino acid residue interactions, the native ligand forms more hydrogen bonds compared to DOXY. Specifically, non-hydrogen residues (Ser105, Val108, Trp84, Ile31, Tyr87, Thr88, Ile288, Tyr92, and Pro285) and hydrogen-bonding residues (Arg167, Tyr35) interact with

Table 1. Top five potential target genes of hypertension and their associated pathways.

Term	*p*-value	Gene
Biological Process		
Regulation of systemic arterial blood pressure by renin-angiotensin (GO:0003081)	4,77E-10	AGTR1, ACE2, AGTR2, REN
Blood vessel diameter maintenance (GO: 0097746)	3,54E-09	AGTR1, ACE2, AGTR2, EDNRA
Regulation of vasoconstriction (GO:0019229)	2,33E-04	AGTR1, ACE2, REN
Regulation of blood sirculation (GO:1903522)	5,34E-04	AGTR1, ACE2, REN
Regulation of reactive oxygen species metabolic process (GO:2000377)	0.000002460	AGTR1, ACE2
Celluler Component		
Secretory granule lumen (GO:0034774)	0.0004318	PLG, C3
Platelet alpha granule lumen (GO:0031093)	0.0004745	C3
Platelet alpha granule (GO:0031091)	0.0008609	PLG
Intracelluler organelle lumen (GO:0070013)	0.007479	PLG, ACE2, REN
Endoplasmic reticulum lumen (GO:0005788)	0.008387	ACE2, C3
Molecular Function		
Endopeptidase activity (GO:0004175)	0.0005121	AGTR1, ACE, ACE2, REN
Transition metal ion binding (GO:0046914)	0.001254	AGTR2, ACE2
Aspartic-type peptidase activity (GO:0070001)	0.006979	EDNRA, AGTR2, ACE2
Aspartic-type endopeptidase Activity (GO: 0004190)	0.008966	PLG, ACE
Phosphatidylinositol phospholipase C activity (GO:0004435)	0.009957	EDNRA

the native ligand, whereas hydrogen bonding at Tyr184 is observed with DOXY. According to the literature, key amino acids in the AGTR1 receptor (PDB ID: 4ZUD), including Arg167, Tyr35, and Trp84, play a pivotal role in ligand recognition. These findings indicate that DOXY and the native ligand bind at the same active site and are predicted to exhibit comparable affinity in inhibiting AGTR1 activity.

Molecular Dynamics Analysis

Molecular dynamics (MD) simulations were conducted for 50 ns to evaluate the stability of the DOXY–AGTR1 complex. The backbone RMSD reached stability after approximately 20 ns and maintained an average deviation of ~3 Å, indicating that the complex attained a stable conformational state and preserved its structural integrity

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

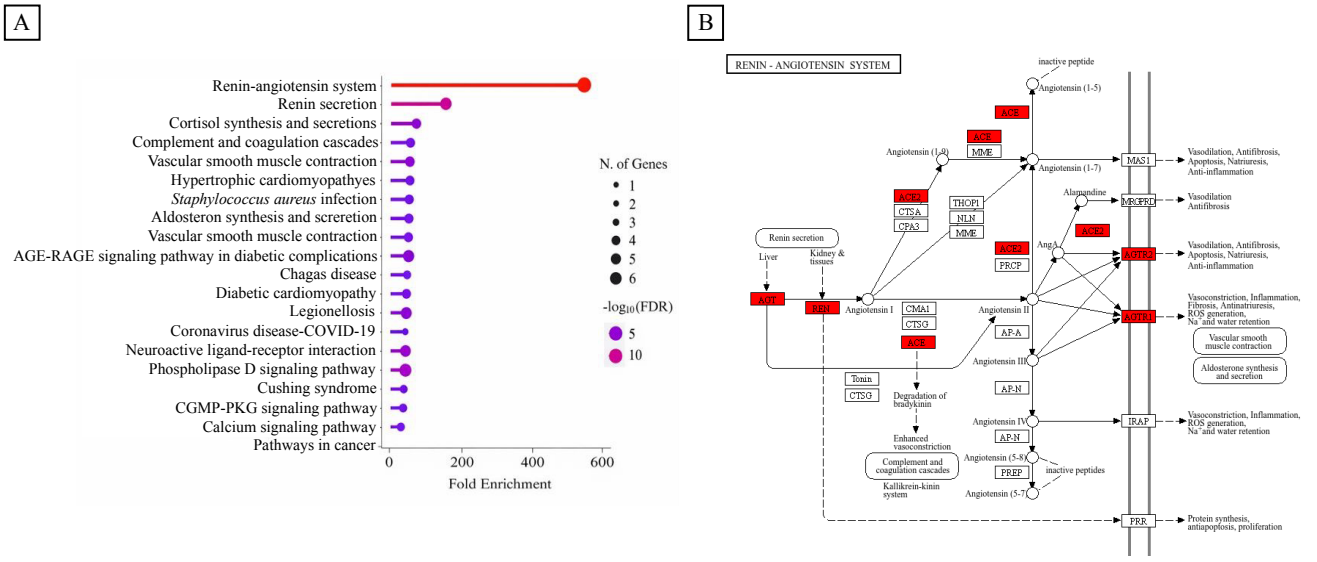


Figure 3. Results of functional enrichment analysis of KEGG pathways. A: Visualization of KEGG pathways based on enrichment. B: Visualization of enrichment results using ShinyGO 0.81. Genes highlighted in red represent the key targets identified in this study that are involved in the renin–angiotensin system and associated with antihypertensive mechanisms.

throughout the simulation (Figure 6A). The root means square fluctuation (RMSF) profile revealed that most amino acid residues exhibited fluctuations below 2 Å, reflecting limited local flexibility. Higher fluctuations were observed in loop regions, which are generally more dynamic, whereas the binding pocket residues remained relatively stable (Figure 6B).

Gene Expression Analysis

The gene expression analysis using qRT-PCR produced cycle threshold (Ct) values, which were reported as the relative expression levels of the target genes compared to an internal control. In this study, the β-actin (ACTB) gene was used as the reference for normalization. The relative expression levels of AGTR1 in kidney tissue were evaluated across three groups: hypertension, DOXY 15 mg/kg BW, and DOXY 30 mg/kg BW. The doxycycline doses of 15 mg/kg and 30 mg/kg were selected based on previous study, who demonstrated that these dose ranges exert pharmacological activity relevant to modulating inflammatory and renin–angiotensin system pathways, making them appropriate for assessing potential antihypertensive effects.13 The hypertensive group showed an average relative expression of 0.31, which decreased to 0.19 in the DOXY 15 mg/kg group, indicating that low-dose doxycycline effectively suppressed AGTR1 expression in kidney tissue. Conversely, the DOXY 30 mg/kg group exhibited a relative expression level of 0.34, slightly higher than the hypertensive group, suggesting a dose-dependent variation in doxycycline's regulatory effect (Figure 7). These results highlight that the 15 mg/kg BW dose of doxycycline demonstrated a stronger inhibitory effect on AGTR1 gene expression in kidney tissue compared to the higher dose.

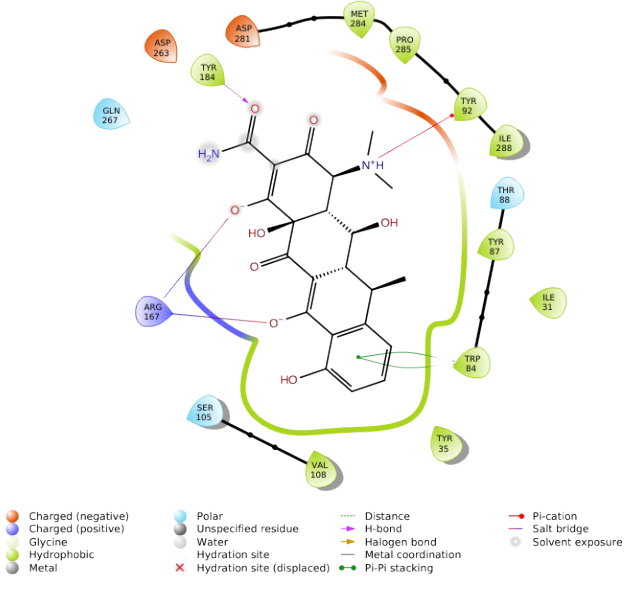


Figure 4. Two-dimensional visualization of amino acid residues involved in the docking interactions of DOXY with AGTR1.

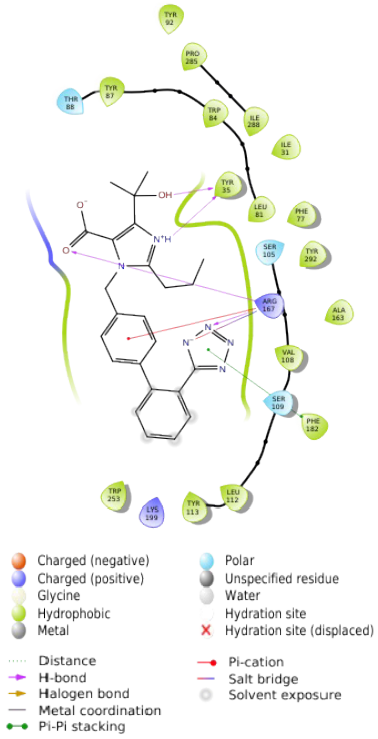


Figure 5. Two-dimensional visualization of amino acid residues involved in the docking interactions of native ligand with AGTR1.

The relative expression levels of AGTR1 in kidney tissue were evaluated across the hypertension, DOXY 15 mg/kg BW, and DOXY 30 mg/kg BW groups. The hypertensive group showed elevated AGTR1 expression (0.31), consistent with the activation of the renin angiotensin system in hypertension. Treatment with doxycycline at 15 mg/kg resulted in a marked reduction of AGTR1 expression (0.19), indicating a potential suppressive effect on vasoconstrictive signaling pathways. However, the 30 mg/kg dose did not further enhance this suppressive effect and instead showed an increased AGTR1 expression (0.34), exceeding the hypertensive group.

Discussion

In this study, doxycycline demonstrated a dose-dependent but non-linear effect on hypertension, with the 15 mg/kg BW dose showing a clearer antihypertensive response compared to the 30 mg/kg BW dose. The reduction in AGTR1 gene expression at 15 mg/kg supports the hypothesis that moderate doxycycline exposure may more effectively modulate the renin–angiotensin system. This finding aligns with recent evidence that doxycycline, beyond its antimicrobial properties, exhibits vascular protective effects through anti-inflammatory and anti-remodeling mechanisms that intersect with RAS signaling.[30,31]

The bioinformatics analysis identified 47 overlapping genes associated with hypertension, with enrichment in pathways central to RAS regulation. This pathway-level coherence strengthens the biological relevance of the selected gene targets. Importantly, our data showed that AGTR1 emerged as a key node, consistent with reports identifying AGTR1 overactivity as a major driver of vasoconstriction, sodium retention, and pro-fibrotic changes in hypertension.[32]

The molecular docking analysis further supported AGTR1 as a plausible target of doxycycline. Doxycycline demonstrated stable binding interactions with critical AGTR1 residues Arg167, Tyr35, and Trp84 which are known to contribute to ligand specificity and receptor

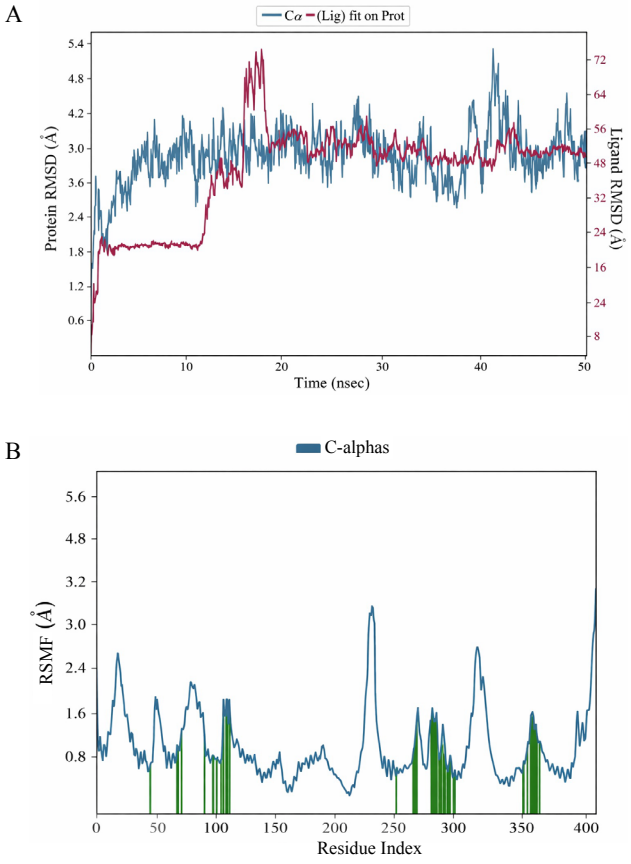


Figure 6. Molecular dynamic analysis. A: RMSD plot of the DOXY–AGTR1 complex showing stabilization after 20 ns. B: RMSF profile indicating low flexibility of most residues, with higher fluctuations in loop regions.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

Table 2. Docking score of doxycycline with native ligand.

Gene	Protein ID	Docking Score		Grid Box		
		Native Ligand	Doxycycline	Grid X	Grid Y	Grid Z
AGT	6RV0	-7.079	-3.328	15.11	09.01	-13.75
AGTR1	4ZUD	-6.295	-8.346	-41.62	63.07.00	28.43.00
AGTR2	5UNH	-9.928	-6.722	105.14.00	-73.85	06.01
REN	2V0Z	-14.075	-5.473	07.57	46.06.00	68.85
ACE	6WF2	-8.424	-3.881	10.22	25.44.00	13.15
ACE2	1R4L	-12.756	-5.388	40.61	0,26528	27.85

activation. Although native ligands exhibited stronger affinity, doxycycline's interaction profile suggests partial antagonistic potential, consistent with recent structural studies indicating that non-classical small molecules can modulate AGTR1 via allosteric or semi-competitive mechanisms.[33-35]

A key finding of this study is the difference in response between the two doxycycline doses. While 15 mg/kg significantly reduced AGTR1 expression, the 30 mg/kg dose resulted in a rebound increase, exceeding hypertensive controls. This paradoxical effect may reflect dose-related shifts in doxycycline pharmacodynamics. Several recent studies have shown that higher doxycycline doses may activate stress-related signaling pathways, increase mitochondrial oxidative burden, or interfere with extracellular matrix remodeling, indirectly diminishing cardiovascular benefit.[36] This pattern suggests that doxycycline's antihypertensive effect may occur within a therapeutic window, beyond which compensatory mechanisms reduce efficacy.

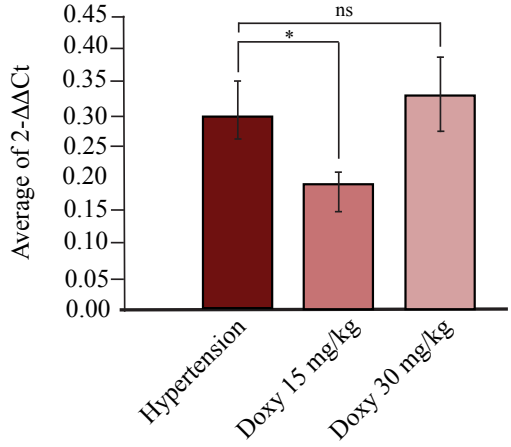


Figure 7. Relative expression levels of AGTR1 genes. Data were presented as mean ± SEM, * $p<0.05$.

The findings imply that doxycycline may influence RAS signaling through both transcriptional and structural mechanisms. Inhibition of AGTR1 has been shown to downregulate PI3K/AKT, ERK/MAPK, and oxidative stress–related pathways, contributing to reduced vasoconstriction and improved endothelial function.[30,37] The expression data from this study are consistent with these mechanistic predictions and help explain the observed blood pressure improvements at the 15 mg/kg dose. Despite these promising findings, several limitations should be acknowledged. This study has several limitations. qRT-PCR analysis was restricted to a limited set of RAS genes, which may constrain pathway-level interpretation; inclusion of additional markers such as AGT, REN, ACE, and ACE2 would strengthen mechanistic conclusions. The modest sample size and use of duplicate technical replicates may affect statistical robustness. Although molecular docking suggested AGTR1 interaction, functional validation at the protein or signaling level was not performed. In addition, blood pressure changes were not quantitatively correlated with molecular outcomes across doses, limiting translational interpretation.

Conclusion

Doxycycline at a dose of 15 mg/kg BW showed a potential antihypertensive effect, although the current data are not

sufficient to conclude a statistically significant reduction in hypertension. The molecular docking and molecular dynamics analyse further indicate that AGTR1 is closely linked to the RAS pathway, supporting its relevance as a therapeutic target. Overall, these findings suggest that doxycycline may modulate key molecular components of hypertensive pathology. However, additional evidence is needed to confirm its antihypertensive efficacy and to fully align the conclusions with the study's broader objectives.

Acknowledgment

The authors would like to express their sincere gratitude to the Head of the Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, for the opportunity to conduct this project. We also extend our appreciation to the Computational Materials Laboratory, Faculty of Engineering, Universitas Indonesia, for providing valuable support and guidance in learning and performing the in-silico analyses.

Authors' Contributions

AF performed data collection, data analysis, laboratory work including both in-silico and in-vitro analyses, and drafted the manuscript. FF and WA contributed to the main concept of the study, validated the data, participated in preparing the final version of the manuscript, supervised the research process, secured funding, and provided critical comments and additional scientific insights. JFF and AFP contributed to the verification and validation of the in-silico data.

Ethical Statement

The study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia (Approval No: KET-353/UN2.F1/ETIK/PPM.00.02/2022).

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1. Jiang Z, Zhai C, Tang G. Novel antihypertensive medications to target the renin-angiotensin system: mechanisms and research. Rev Cardiovasc Med. 2025; 26(4): 27963. doi:10.31083/RCM27963.
2. Ramadhanti R, Helda H. Association of hypertension and chronic kidney disease in population aged ≥18 years old. Mol Cell Biomed Sci. 2021; 5(3): 137-44.
3. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, *et al*. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiol Rev. 2018; 98(3): 1627-738.
4. Alam R, Ahsan H, Khan S. The role of malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP) in patients with hypertension. Mol Cell Biomed Sci. 2023; 7(2): 58-64.
5. Martyniak A, Drożdż D, Tomasik PJ. Classical and alternative pathways of the renin–angiotensin–aldosterone system in regulating blood pressure in hypertension and obese adolescents. Biomedicines. 2024; 12(3): 620-30.
6. Bader M, Steckelings UM, Alenina N, Santos RAS, Ferrario CM. Alternative renin-angiotensin system. Hypertension. 2024; 81(5): 964-76.
7. Garcia B, Zarbock A, Bellomo R, Legrand M. The alternative renin–angiotensin system in critically ill patients: pathophysiology and therapeutic implications. Crit Care. 2023; 27(1): 453. doi: 10.1186/s13054-023-04739-5.
8. Safitri PGA, Da'i M, Wulandari F. 1'-Acetoxychavicol acetate suppresses osteosarcoma cell proliferation through the PI3K pathway: a molecular docking and cytotoxicity study. Mol Cell Biomed Sci. 2025; 9(2): 105-14.
9. Taherkhani S, Sheibani M, Mohammadkhanizadeh A, Virag JAI, de Castro Braz L, Azizi Y. Metalloproteinases (MMPs) in hypertensive disorders: role, function, pharmacology, and potential strategies to mitigate pathophysiological changes. Front Pharmacol. 2025; 16: 1559288. doi: 10.3389/fphar.2025.1559288.
10. de Oliveira NVG, Blascke MMM, Rodrigues D, Pernomian L, de Oliveira IS, Parente JM, *et al*. Type I collagen proteolysis by matrix metalloproteinase-2 contributes to focal adhesion kinase activation and vascular smooth muscle cell proliferation in the aorta in early hypertension. Vascul Pharmacol. 2023; 152: 107211. doi: 10.1016/j.vph.2023.107211.
11. Blascke de Mello MM, Neves VG O, Parente JM, Pernomian L, de Oliveira IS, Pedersoli CA, *et al*. Sarcoplasmic reticulum calcium ATPase (SERCA) proteolysis by matrix metalloproteinase-2 contributes to vascular dysfunction in early hypertension. Eur J Pharmacol. 2024; 983: 176981. doi: 10.1016/j.ejphar.2024.176981.
12. Robles-Vera I, de la Visitación N, Toral M, Sánchez M, Romero M, Gómez-Guzmán M, *et al*. Changes in gut microbiota induced by doxycycline influence in vascular function and development of hypertension in DOCA-salt rats. Nutrients. 2021; 13(9): 2971. doi: 10.3390/nu13092971.
13. Monayo ER, Arozal W, Noviana D, Barinda AJ, Widyantoro B. Sprague-dawley rats induced by uninephrectomy, DOCA injection, and sodium chloride: a suitable model for chronic hypertension with cardiac hypertrophy. Int J Vet Sci. 2024; 13(5): 623-31.
14. Davis AP, Wiegers TC, Johnson RJ, Sciaky D, Wiegers J, Mattingly CJ. Comparative Toxicogenomics Database (CTD): update 2023. Nucleic Acids Res. 2023; 51(D1): D1257-62.
15. Berthier CC, Bethunaickan R, Gonzalez-Rivera T, Nair V, Ramanujam M, Zhang W, *et al*. Cross-Species Transcriptional Network Analysis Defines Shared Inflammatory Responses in Murine and Human Lupus Nephritis. J. Immunol. 2012; 189(2): 988-1001.
16. Guo F, Zhang W, Su J, Xu H, Yang H. Prediction of drug positioning for quan-du-zhong capsules against hypertensive nephropathy based on the robustness of disease network. Front Pharmacol. 2019; 10: 49. doi: 10.3389/fphar.2019.00049.
17. Chen Z, Wu H, Wang G, Feng Y. Identification of potential candidate

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v10n1.744

genes for hypertensive nephropathy based on gene expression profile. BMC Nephrol. 2016; 17(1): 1-8.
18. Bioinformatics & Evolutionary Genomics [Internet]. Calculate and draw custom Venn diagrams [updated 2025 January 12; cited by 2025 August 30]. Available from: https://bioinformatics.psb.ugent.be/webtools/Venn/.
19. Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, *et al*. The STRING database in 2025: protein networks with directionality of regulation. Nucleic Acids Res. 2025; 53(D1): D730–7.
20. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape stringapp: network analysis and visualization of proteomics data. J Proteome Res. 2019; 18(2): 623-32.
21. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. CytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014; 8(Suppl 4): S11. doi: 10.1186/1752-0509-8-S4-S11.
22. Evangelista JE, Xie Z, Marino GB, Nguyen N, Clarke DJB, Ma'Ayan A. Enrichr-KG: bridging enrichment analysis across multiple libraries. Nucleic Acids Res. 2023; 51(1W): W168–79.
23. Xie Z, Bailey A, Kuleshov MV, Clarke DJB, Evangelista JE, Jenkins SL, *et al*. Gene set knowledge discovery with enrichr. Curr Protoc. 202; 1(3): e90. doi: 10.1002/cpz1.90.
24. Xijin Ge S, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 2020; 36(8): 2628-9.
25. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Costanzo L Di, *et al*. Protein data bank: the single global archive for 3d macromolecular structure data. Nucleic Acids Res. 2019; 47(D1): D520–8.
26. National Center for Biotechnology Information [Internet]. PubChem compound summary for CID 54671203, Doxycycline [cited 2025 January 12]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Doxycycline.
27. Sharma J, Kumar BV, Singh R, Rajendran V, Purohit R, Kumar S. An in-silico evaluation of different bioactive molecules of tea for their inhibition potency against non-structural protein-15 of SARS-CoV-2. Food Chem. 2021; 346: 128933. doi: 10.1016/j.foodchem.
28. Wagner EM. Monitoring gene expression: Quantitative real-time RT-PCR. Methods Mol Bio. 2013; 1027: 19–45.
29. Jozefczuk J, Adjaye J. Quantitative real-time PCR-based analysis of gene expression. In: Methods Enzymol. 2011; 500: 99-109.
30. Zeng X, Yang Y. Molecular mechanisms underlying vascular remodeling in hypertension. Rev Cardiovasc Med. 2024; 25(2): 72. doi: 10.31083/j.rcm2502072.
31. Robles-Vera I, de la Visitación N, Toral M, Sánchez M, Romero M, Gómez-Guzmán M, *et al*. Changes in gut microbiota induced by doxycycline influence in vascular function and development of hypertension in DOCA-salt rats. Nutrients. 2021; 13(9): 2971. doi: 10.3390/nu13092971.
32. Chen S, Li B, Chen L, Jiang H. Uncovering the mechanism of resveratrol in the treatment of diabetic kidney disease based on network pharmacology, molecular docking, and experimental validation. J Transl Med. 2023; 21(1): 380. doi: 10.1186/s12967-023-04233-0.
33. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics data integration, interpretation, and its application. Bioinform Biol Insights. 2020; 14: 1177932219899051. doi: 10.1177/1177932219899051.
34. Sayed Murad HA., M Rafeeq M, Alqahtani SM, S Rajab B, Alghamdi S, J Almehmadi S, *et al*. Molecular docking analysis of AGTR1 antagonists. Bioinformation. 2023; 19(3): 284-9.
35. Zeng Y, Jiang Y, Huang Z, Li K, Zhou Y. Association between AGTR1 (c.1166 A>C) polymorphisms and kidney injury in Hypertension. Front Biosci. 2023 ;28(7): 146. doi: 10.31083/j.fbl2807146.
36. Guimaraes DA, Rizzi E, Ceron CS, Oliveira AM, Oliveira DM, Castro MM, et al. Doxycycline dose-dependently inhibits MMP-2-mediated vascular changes in 2K1C hypertension. Basic Clin Pharmacol Toxicol. 2011; 108(5): 318–25.
37. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, et al. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiol Rev. 2018; 98(3): 1627–738.