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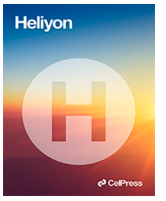

Research article

Mechanism of Mongolian mind-body interactive therapy in regulating essential hypertension through HTR2B: A metabolome- and transcriptome-based study

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ABSTRACT

Essential hypertension is a psychosomatic disease associated with emotions and behaviors. Although Mongolian mind–body interactive therapy can help patients with essential hypertension reduce their systolic blood pressure (SBP), the mechanism is unclear. We assigned patients who underwent Mongolian mind–body interactive therapy to groups that were treated with (DT) or without (NDT) antihypertensive drugs (Clinical registration no: ChiCTR2000034918). We screened differentially expressed genes (DEGs) using targeted metabolic and transcriptomic analyses of blood samples before and after intervention. Sequenced data were analyzed using quantitative polymerase chain reaction (qPCR) and validated using enzyme-linked immunosorbent assays (ELISAs). Small interfering (Si)-RNA interference on key DEGs in human umbilical vein endothelial cells (HUVECs) was experimentally verified. Omics analysis identified 187 DEGS, including human 5-hydroxytryptamine (5-HT) receptor 2B (5-*HTR2B*), human endothelin receptor type B (*EDNRB*), and the metabolite N-acetylserotonin. The qPCR and transcriptome sequencing results were consistent. Post-intervention ELISA assays revealed significantly elevated 5-HT in the NDT group after intervention ($P < 0.05$). Interactions between 5-*HTR2B* and N-acetylserotonin differed between the groups. The cellular findings showed significantly reduced G protein-coupled receptor 82 (GPR82) and phospholipid phosphatase-related protein type 4 (PLPPR4), and significantly increased S100A2 protein expression in the Si-HTR2B group, compared with the controls ($P < 0.05$). The biochemical results uncovered significantly decreased nitric oxide (NO) and significantly increased malondialdehyde and NO synthetase concentrations compared with the models ($P < 0.05$). Mongolian mind–body interactive therapy might affect SBP in patients with essential hypertension by combining 5-HT with 5-*HTR2B* to mediate NO relaxation.

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1. Introduction

Essential hypertension (EH) affects over a billion individuals globally and is among the leading contributors to mortality from cardiovascular disease [1]. The 2022 Chinese Guidelines for the Management of Hypertension and the American College of Cardiology/American Heart Association Hypertension Guidelines define WH as a persistent systolic blood pressure (SBP) of ≥130 mmHg and/or diastolic blood pressure (DBP) of ≥80 mmHg. While immediate pharmacological intervention might not be appropriate for most patients with blood pressure 130–139/80–89 mmHg, novel therapeutic approaches should be explored [2]. Antihypertensive drugs are currently the primary method of hypertension management. However, lifestyle and psychological interventions have been recommended as adjunct therapies that might help to reduce or prevent the adverse effects of drugs. Consequently, research into non-pharmacological interventions for mild-to-moderate EH is increasing. Numerous randomized controlled trials and pairwise meta-analyses have assessed approaches to lower blood pressure such as dietary changes aerodynamic exercise, isometric training, comprehensive lifestyle modification, breathing control, and other non-pharmacological therapies [3]. Preliminary results from an 8-week mindfulness intervention in the USA suggest that these approaches are effective. Additionally, diffusion tensor magnetic resonance imaging (DT-MRI) has revealed that mindfulness interventions induce modifications in structural brain connectivity, which might mediate beneficial changes in internal sensory awareness and depression in patients with hypertension [4].

Many classical works of Mongolian medicine describe that black veins contain blood, "Xira" storage relies on blood, and black vein disease should be classified as "Rezheng" [5,6]. The causes of this disease are believed to be excessive worry, physical and mental exhaustion, excessive excitement, emotional discomfort, anger, prolonged excessive alcohol consumption, smoking, a high-cholesterol diet, lack of physical activity, and other reasons [7]. Mongolian medicine classifies hypertension as "black vein disease", which is closely linked to psychological and emotional factors. Mongolian mind–body interactive therapy (MMBIT) is a psychosomatic treatment that incorporates elements of local Mongolian culture. It comprises music, health preservation, case reports, and psychosomatic treatment. It was founded on the unified theory of mind–body in Mongolian medicine and modern medical psychology principles and methods [8]. Mongolian mind–body interactive therapy primarily regulates the nervous system and immunity and uses a health education platform to facilitate psychological communication [9]. Patients generally acknowledge its cost-effectiveness and absence of adverse effects, and it has been remarkably effective in treating psychosomatic disorders induced by social and psychological determinants [9,10].

We aimed to identify the effects of MMBIT on patient blood pressure by analyzing pre- and post-intervention data. We also explored the roles of key factors during MMBIT and possible mechanisms of action, to provide a scientific basis for the outcomes of MMBIT.

2. Materials and methods

2.1. Study design

Twenty patients with EH who underwent a 28-day MMBIT intervention were assigned to groups treated with (DT) or without (NDT) oral dipine antihypertensive drugs according to the recommendations of an attending physician to clarify the effects of MMBIT on EH (see Fig. 1). This clinical study was listed in The Clinical Trial Registry on 2020-07-24 under the title, "Self-control study of Mongolian

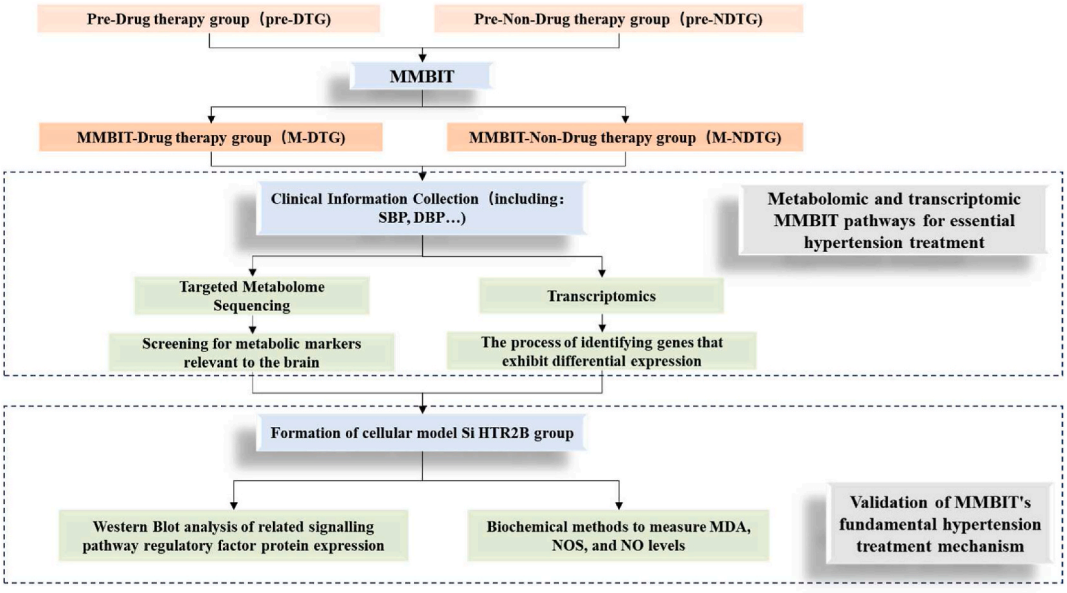

Fig. 1. Study framework.

Mind-Body Interactive Psychotherapy in the Treatment of Essential Hypertension" (No: ChiCTR2000034918).

2.2. Participants

Blood samples were obtained from patients with EH who were admitted to the International Mongolian Medical Hospital of the Inner Mongolia Autonomous Region between January 2022 and December 2022. This study proceeded according to the ethical principles enshrined in the Declaration of Helsinki (2013 amendment) and the International Ethical Guidelines for Biomedical Research in Human Beings published by the International Medical Science Organization Committee. The Ethics Committee at International Mongolian Medicine Hospital approved this study (Approval No: 2020-014). The patients were informed about the aims and procedures of the study before they provided written informed consent to participate. The inclusion criteria comprised: meeting the definition of EH according to the 2018 Chinese Guidelines for the Management of Hypertension, mild to moderate hypertension, and age 18–70 years. The exclusion criteria comprised cardiac diseases such as sick sinus syndrome or aortic stenosis, serious dysfunction of the heart, brain, and kidneys, and damage to the circulatory system due to high blood pressure.

2.3. Therapy details

Mongolian mind–body interactive therapy is a comprehensive approach that essentially combines health education with Mongolian songs, health exercises, and narrative therapy. Group therapy is applied to 200–1000 persons per session depending on the size of the treatment room. Narrative therapy mainly introduces the clinical maladies and symptoms of participants and how they can be overcome, and how to improve self-awareness after MMBIT *via* case studies. Experienced physicians comment on all case reports. Patients only need to feel and receive a sense of relaxation, regardless of whether treatment is applied to a specific population. Details of intervention using MMBIT are described elsewhere [9,10].

2.4. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

We detected 39 neurotransmitters in serum using high-performance liquid chromatography using tandem mass spectrometry (HPLC-MS/MS) and quantified them by comparison with standard curves prepared by shaking a gradient dilution of 39 neurotransmitters in pyridine at 25 °C for 30 min followed by centrifugation. Serum samples were analyzed using a Waters I class -AB Sciex QTRAP 6500 (Sciex, Framingham, MA, USA) on a BEHC18 column (model: 1.7 μm*2.1*100 mm (Waters Corp., Framingham, MA, USA). The mobile phases A and B comprised 0.1 % formic acid in water and 0.1 % formic acid in methanol, respectively. The flow rate was 0.35 mL/min and the gradient was 2 % A for 0–2 min, 20–80 % B for 2.5–15 min. Default settings in Analyst software (SCIEX) to automatically identify and integrate each Multiple Reaction Monitoring (MRM) transition (ion pair). Metabolites were screened using VIP $\geq$1, fold change $\geq$1.5 or $\leq$0.667 in a Partial Least Squares Discriminant Analysis (PLS-DA) model, principal component analysis (PCA), and one-way analysis of variance (ANOVA). Values with $P < 0.05$ were considered statistically significant.

2.5. Transcriptome sequencing and analysis

Total RNA was extracted from leukocytes in whole blood using UltraPure RNA Extraction Kits (CW0581M; CWBIO, Cambridge, MA, USA) and quantified to construct an mRNA library. After sequence filtering and alignment, Fragments Per Kilobase per million (FPKM) exons were computed using StringTie and TMM to analyze gene expression, and fold changes (FCs) in differential expression were determined. Genes were selected based on $P < 0.05$ and $FC > 1$. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) enrichment were analyzed using the Dr. Tom system (BGI Genomics, Yantian, China).

2.6. Association analysis of metabolome and transcriptome

Univariate, unsupervised, and supervised models and biological function associations were analyzed. The top 20 most closely correlated pairs of differentially expressed genes (DEGs) and metabolites were selected by analyzing a correlation matrix visualized as a network diagram.

2.7. Validation by q-PCR and ELISA

We validated the RNA sequencing (RNA-seq) data using qPCR and with the primers for insulin-like growth factor 1 (*IGF-1*), *HTR1B*, *HTR2B*, *S100A2*, *EDNRB*, Monoamine Oxidase B (*MAO-B*), and G-protein subunit alpha 14 (*GNA14*) listed in Supplementary Table 1. Expression was normalized to that of the reference gene GAPDH. We reverse-transcribed RNA (1 μg) using HiScript IIQ RT SuperMix for qPCR Reagent Kits (Vazyme, Nanjing, China) as described by the manufacturer. Targeted DNA sequences were amplified by qPCR using ChamQ Universal SYBR qPCR Master Mix (Vazyme). Serum levels of 5-HT were quantified using serotonin/5-HT (ST/5-HT) ELISA kits (D751013; BBI Solutions, Portland, ME, USA) as described by the manufacturer.

2.8. Cell validation

Human Umbilical Vein Endothelial Cells (HUVECs) (ATCC, Manassas, VA, USA) were stimulated with angiotensin II (Ang II) (1

μmol/L) for 24 h to create a model of damage due to hypertension. We also designed small interfering (Si-RNA) targeting HTR2B (Si-HTR2B) to inhibit the model (Supplementary Table 2). Normal HUVECs (controls) were incubated with angiotensin II (model) which was then transfected with plasmid (NC), or induced with Si-HTR2B and nifedipine (NI). The expression of PLPPR4, GPR82, HTR2B, and S100A2 proteins was determined by western blotting. Blotted proteins were analyzed using Image J software (NIH, Bethesda, MD, USA) with the grey value of GADPH serving as the internal control. Relative expression was determined by dividing the grey values of target proteins by that of GADPH. Concentrations of nitric oxide (NO), nitric oxide synthase (NOS), and malondialdehyde (MDA) were respectively determined using the following test kits, A012-1-2 and A014-2-2 (Nanjing Jianjian, Nanjing, China), and E-EL-0060c (Eli Lilly, Indianapolis, IN, USA).

2.9. Data analysis

Data are presented as means ± standard deviations. Graphs were generated using GraphPad Prism v. 8.0 (GraphPad Software, La Jolla, CA, USA). Disparity between actual and predicted data was determined using chi-square (χ^2) tests and pairs of groups were compared using nonparametric rank-sum tests. All data were statistically analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA Values with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of the participants

Table 1 shows that 90 % and 80 % of patients in the DT and NDT groups (n = 10 each) had stable and unstable blood pressure, respectively. Other characteristics were comparable between the groups. The SBP decreased in both groups after 28 days of MMBIT intervention and from 153.4 to 148 mmHg in the NDT group.

3.2. Targeted metabolomics and transcriptome findings

We sequenced and analyzed metabolomes in blood samples from the DT and NDT groups before and after intervention (Supplementary Figs. 1A and B). The metabolome analyzed based on the PCA results of the DT group showed that PC1, PC2, and PC3 respectively accounted for 18.98 %, 14.31 %, and 10.77 % of the variation. The PCA of the NDT group showed that PC1, PC2, and PC3 accounted for 20.56 %, 14.31 %, and 10.77 %, respectively. The results indicated tight sample clustering (Fig. 2A and B) and N-acetylserotonin (NAS) was detected. Differential metabolites were enriched in the metabolic pathways of formyl-N-acetyl-5-methoxycoumarinamine and 6-hydroxymelatonin. These results indicated a significant association between NAS and serotonin metabolic pathways.

Transcriptome sequencing after intervention identified 92 (38 upregulated and 54 downregulated) DEGs in the DT group and 95 (43 upregulated and 52 downregulated) DEGs in the NDT group, (Fig. 2C and D; Supplementary Figs. 2A and B).

We selected *IGF-1, HTR1B, HTR2B, S100A2, EDNRB, MAO-B,* and *GNA14* for q-PCR validation (Fig. 2E). The 5-HT levels in groups were analyzed using an ELISA. In the NDT group, The 5-HT values were significantly higher after intervention in the NDT group ($P < 0.05$) but were unchanged in the DT group (Fig. 2F and G).

3.3. Associations of transcriptome and metabolome findings

Metabolomic analysis of a specific set of metabolites associated with the parameters that varied in the transcriptome revealed that NAS plays a role in the serotonin anabolic pathway (Fig. 3A). The neurotransmitter 5-HT interacted with G0/G1 switch 2 (*GOS2*) and RAB3A interacting protein like 1 (*RAB3IL1*), whereas NAS metabolites interacted with (*IGF-1*), Proline Rich 36 (*PRR36*), and Bublin Coiled Coil Protein (*C9orf16*) in the NDT group We also found that 5-HT was associated with Carbonic Anhydrase 1 (*CA1*), Tre-2/Bub2/Cdc (*TBC*), Selenium Binding Protein 1(*SELENBP1*), and hemoglobin subunit zeta (*HBZ*). In the DT group, NAS metabolites interacted with protein tyrosine phosphatase receptor type H (*PTPRH*) and amphiphysin (*AMPH*) (Fig. 3B and C).

3.4. Effects of Si-HTR2B on HUVECs

The ideal concentration of Ang II required to induce the model was 2 μM (Supplementary Material Fig. 3A and B). Western blots revealed significantly lower PLPPR4 and GPR82 protein expression in the Si-HTR2B and NI groups than in the controls; further, the expression was lower in the Si-HTR2B group than in the NC group. The expression of 5-hydroxytryptamine receptor 2B (HTR2B) protein significantly differed between the NC and control groups, but not between the other sand control groups. Additionally, S100A2 protein expression was more abundant in the Si-HTR2B, Model, and NC groups than in the controls (Fig. 4A).

Biochemical index assays revealed more elevated MDA levels in the model, than in the controls, but decreased in the Si-HTR2B and NI groups (Fig. 4B). The amount of NOS activity was considerably higher in the model than in the control and NI groups (Fig. 4C). The NO concentration was significantly higher in the model than the control, but lower in the Si-HTR2B group than in the model (Fig. 4D).

Table 1
Baseline characteristics of participants.

	Sex		Age(y)	History of familial hypertension	Stable blood pressure	Hypertension grade			Hypertension risk stratification		Medical complication		Monglian hypertension grading			Week1/4			
	Male	Female	-	-	-	I	II	III	Moderate	Elevated	Yes	No	BT	BH	H	SBP (mmHg)	DBP	SBP	DBP
NDT	3/10	7/10	49.3 ± 8.98	6/10	2/10	2/10	7/10	1/10	9/10	1/10	2/10	8/10	3/10	5/10	2/10	153.4/148	97.9/89.5	-	-
DT	4/10	6/10	54.2 ± 5	7/10	9/10	2/10	6/10	2/10	9/10	1/10	5/10	5/10	4/10	2/10	4/10	-	-	133.5/129.6	89.5/77.4
P	0.639		0.6137	0.639	0.001*	0.814			0.999		0.159		0.350			0.027†	0.232	0.275	0.105
χ^2	0.219		-	0.219	9.899	0.410			0		1.978		2.095			-	-	-	-

*$p < 0.001$, †$p < 0.05$. DBP, diastolic blood pressure; SBP, systolic blood pressure; Batakan deviant,BT; Blood Hira deviant,BH; Hei deviant, H.

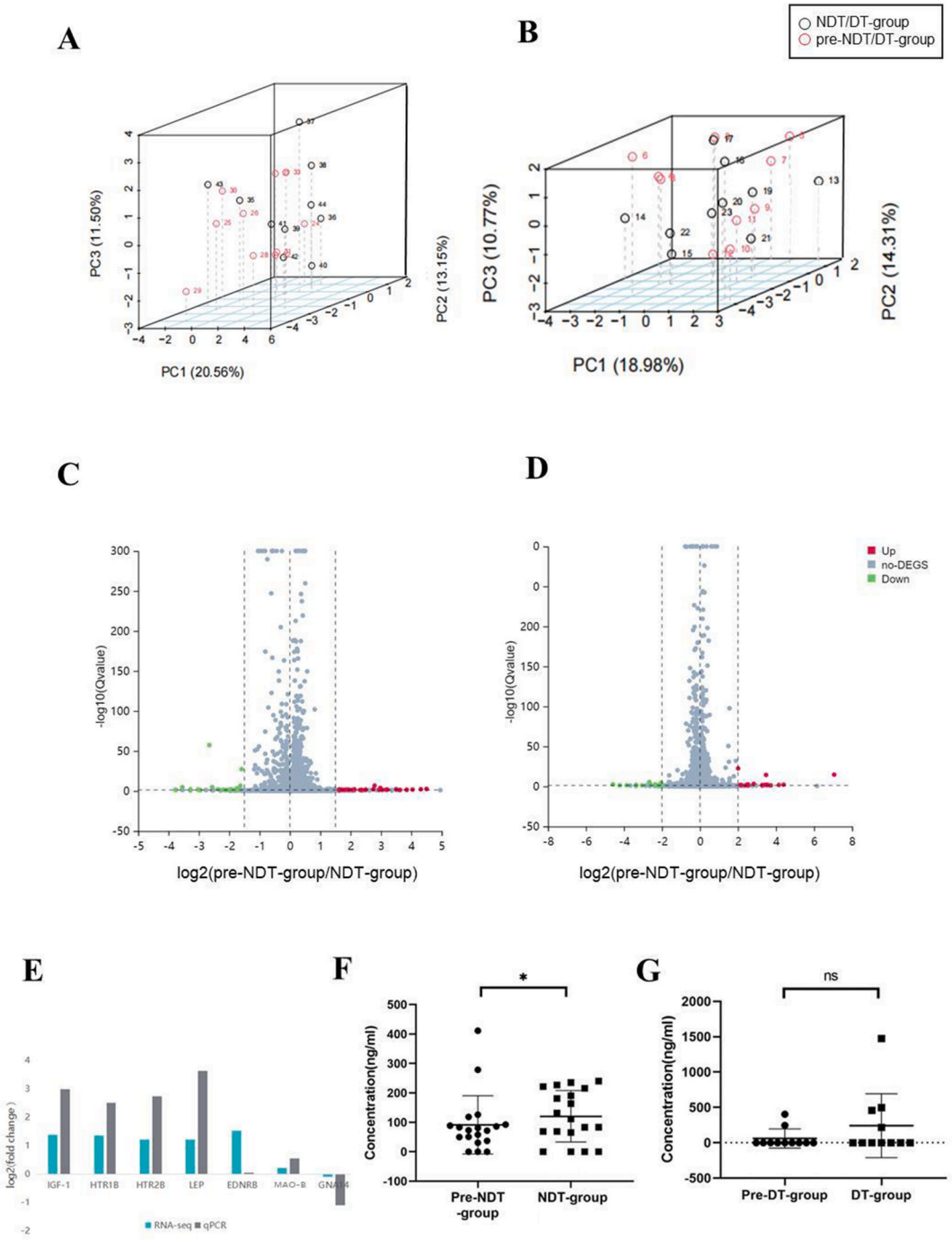

Fig. 2. Expression of targeted metabolome and transcriptome pre- and after- MMBIT intervention in EH.
(A,B) Plots of PCA-targeted metabolic data in the NDT and DT groups (n = 10 each). Red and black indicate before and after intervention, respectively. (C,D) Volcano maps of DEGs in transcriptomes of both groups. (E) Trends of seven DEGs analyzed by qPCR *vs.* RNA-seq (n = 19). (E-G) Results of 5-HT ELISA in DT (n = 11) and NDT (n = 19) groups. ns: no difference. *P < 0.05. Down, downregulated genes; DT, drug therapy; NDT, no drug therapy; no DEGs, undifferentiated genes; up, unregulated genes.

3.5. 5-HT-binding HTR2B receptor signaling pathway increases endothelial NO release

Based on the transcriptome and metabolic findings in the DT and NDT groups, we screened candidate genes using qPCR and ELISA and validated the transcriptome data using experiments *in vitro*. The biological effects might be driven by regulating SBP pathways in

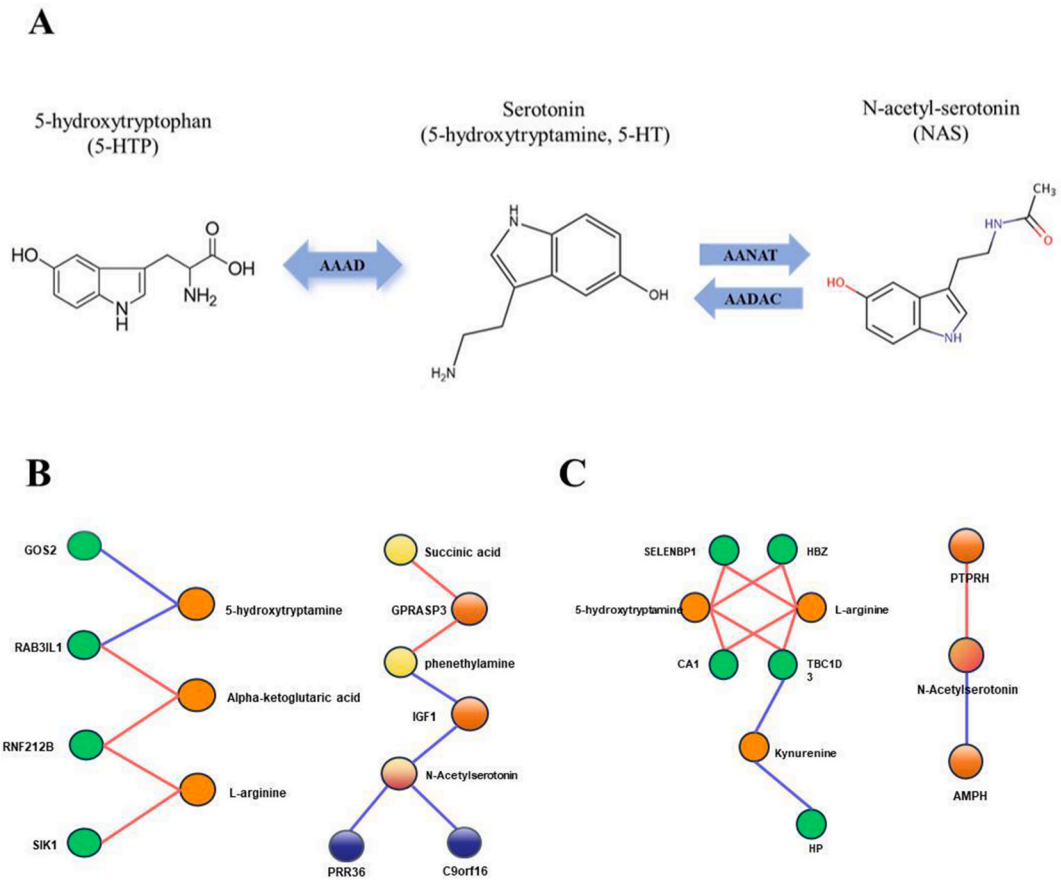

Fig. 3. Spearman relevance network connecting DEGs and metabolites.
(A) Anabolic serotonin pathway. (B) Interaction between DEGs with metabolites in the NDT group. (C) Interaction between DEGs with metabolites in DT group. Green and purple represent transcriptome DEGs and yellow and orange represent target metabolites. AAAD, aromatic L-amino acid decarboxylase; AADAC, arylacetamide deacetylase AANAT; aralkylamine-N-acetyltransferase.

patients with primary hypertension who underwent MMBIT. Brain stimulation can lead to elevated levels of 5-hydroxytryptophan (5-HTP) which subsequently crosses the blood-brain barrier, resulting in increased blood 5-HT concentrations. The binding of 5-HT to HTR2B in vascular epithelial cells activates the G protein-coupled receptor (GPCR) signaling pathway, which ultimately leads to increased levels of NO, which exerts vasodilatory effects (Fig. 5).

4. Discussion

Hypertension is usually managed by antihypertensive medications, the effectiveness of which varies depending on the cause of hypertension [11]. The vasculature independently secretes substances that affect physiological functions, and the modified physical properties of the vasculature affect blood pressure. This pathophysiological phenomenon is involved in the blood pressure-lowering effects of vasoactive intestinal peptide receptor agonists [12,13]. The renin-angiotensin-aldosterone system is crucial for maintaining fluid balance and controlling blood pressure over time and renin and aldosterone receptor antagonists are the most prevalent medications targeting this system [14]. Nitric oxide is a powerful vasodilator that helps regulate and stabilize blood pressure [15]. Endothelium located at the boundary between blood circulation and vessels detects changes in blood flow, pressure, inflammation, numerous hormones, and other signals in the bloodstream. Renal water and sodium reabsorption promoted by increased blood insulin levels leads to an increase in effective circulating blood volume and a subsequent increase in blood pressure. Therefore, insulin-sensitive medications can be administered [16]. The autonomic nervous system regulates the heart and as well as blood vessel activity by releasing catecholamines from the sympathetic nerves. Sympathetic excitation significantly increases during the early stages of hypertension. This suggests that abnormal neurohormone regulation plays a crucial role in the development and progression of hypertension. Dopamine β-hydroxylase inhibitors can lower blood pressure in this context [17]. Psychological variables are essential for high blood pressure and affect all aspects of its development. Patients who underwent MMBIT experienced a shift from negative emotions, including pessimism, anger, and disappointment, to positive emotions, such as exuberance, happiness, and hope. They experience various physical sensations that indicate comfort and improved clinical findings. Managing psychological states might help

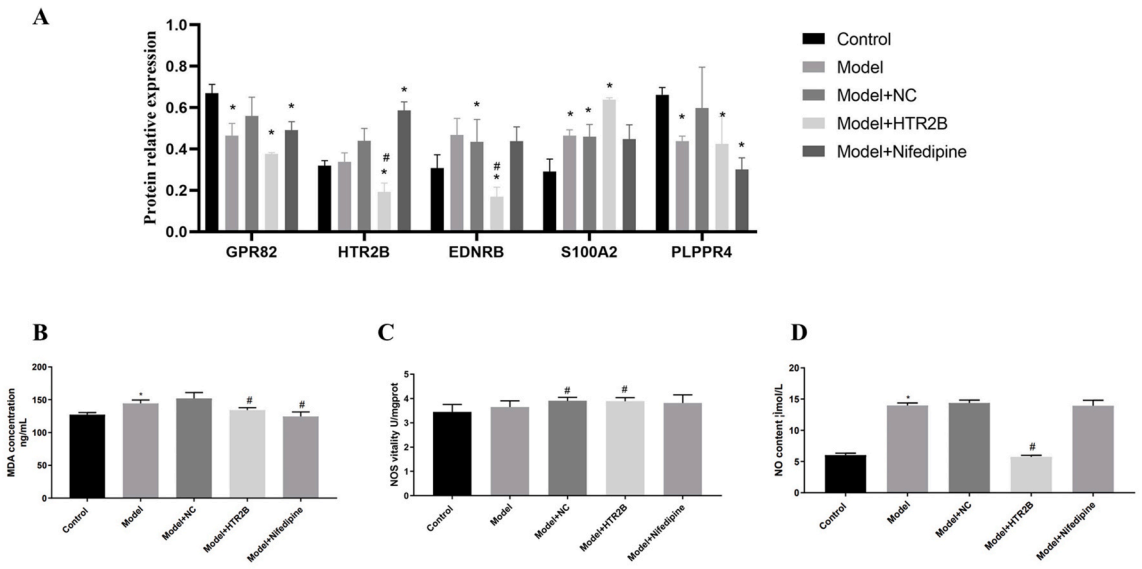

Fig. 4. Effects of Si-HTR2B on MDA, NOS, and NO.
(A) Protein expression of PLPPR4, GPR82, HTR2B, and S100A2 in models *in vitro* (B–D) Impact of the HTR2B intervention on MDA, NOS, and NO values in the model. *P < 0.05 *vs.* control; †P < 0.05 *vs.* model. GPR82, G protein-coupled receptor 82; HTR2B, 5-hydroxytryptamine receptor 2B; NO, nitric oxide; NOS, nitric oxide synthase; PLPPR4, phospholipid phosphatase related 4.

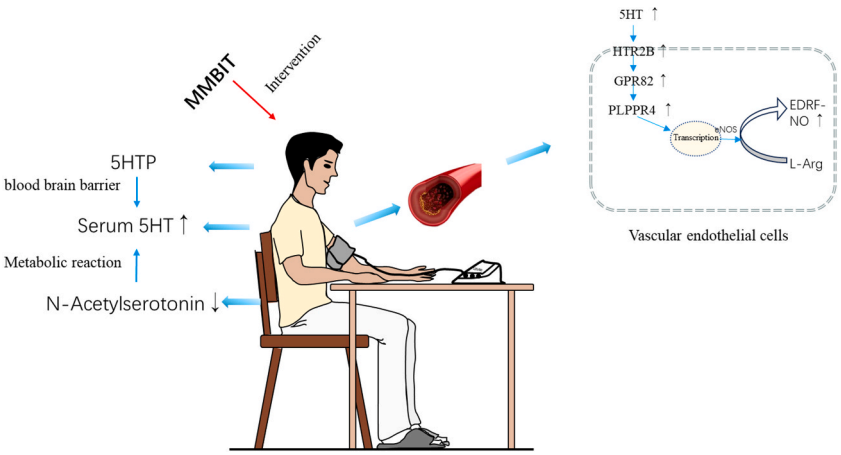

Fig. 5. Intervention with MMBIT uses a mechanistic model of 5-HT to treat mild EH.

to improve physical wellbeing [18,19]. The baseline characteristics of our participants were comparable. However, intervention with MMBIT was associated with more unstable blood pressure in the NDT group. This might have been due to Not take antihypertensive drug regimens. One week after intervention, The SBP of patients in the NDT group significantly decreased by one week after MMBIT.

The expression of RNA might reflect physiological changes associated with hypertension, suggesting that transcriptomic research has clinical applications. Altered RNA expression is reflected in physiological changes associated with hypertension [20]. We identified eight distinct genes that were collectively associated with variations in blood pressure and could be used as biomarkers to diagnose hypertension. A genome-wide association study (GWAS) of samples from patients in Europe using shingled magnetic recording (SMR) validation, and Unified Test for MOlecular SignaTures (UTMOST), associated the potassium two pore domain channel subfamily K member 3 (KCNK3), Glutamyl Aminopeptidase (ENPEP), and Ubiquitin Specific Peptidase 38 (USP38) genes with hypertension. That study explored the association between hypertension traits at the genetic level and targeted genes more accurately than previous single nucleotide polymorphism (SNP) studies [21]. A randomized, controlled, single-blind study using traditional Liuzijue transfer found that IL-6 and IL-10 expression increased after intervening 12 weeks in hypertension patients, and tended to have a different gut microbiota structure from controls [22]. That study showed that the brain-gut axis in patients with hypertension can be changed by traditional therapy, which might be one mechanism of its action. Moreover, aerobic training as a control group can more scientifically show liuzijue's unique advantages. The results of RNA sequencing have shown that the traditional Chinese drug, after taking

QiShenYiQiDi pill, enhances the expression of salt inducible kinase 1 (SIK1) promotes Na^+ excretion, and suppresses adrenoceptor alpha 1A (ADRA1D) expression. These changes are associated with decreased blood pressure in salt-sensitive hypertensive rats. This study aimed to elucidate the mechanism through which this drug lowers blood pressure [23]. Others have investigated gene expression using transcriptomics in hypertensive rats after acupuncture. These findings revealed that acupuncture reduces blood pressure by modulating DEGs associated with hypertension-related pathways [24].

Metabolomics can determine physiological activities by analyzing changes in metabolites resulting from external stimuli or the internal milieu [25]. The overall metabolomic profile of serum and urine samples obtained from hypertensive mice revealed 14 and 6 substantially altered metabolites, respectively [26]. Huanglian-derived polysaccharides promote the growth of beneficial bacteria within the gut microbiota and increase the concentrations of short-chain fatty acids, particularly butyric acid [27]. Metabolomic findings have suggested that cetylenedioate is linked to hypertension in humans, and transcriptomic findings have associated its metabolism with the levels of Cytochrome P450 4 t(*CYP4*) and Alcohol dehydrogenase 1A (*ADH1A*) enzymes, which are encoded by the fat ω-oxidation pathway [28]. The relationship between blood pressure and plasma metabolites has been investigated using metabolomics [29]. The results showed that ceramide, triacylglycerol, total triglyceride, and oleic acid levels positively correlate, whereas cholesterol lipids negatively correlate with DBP. In summary, transcriptomics and metabolomics can help to elucidate the mechanisms underlying hypertension.

Here, we applied targeted metabolome and transcriptome analyses to blood samples from the DT and NDT groups before and after undergoing MMBIT. Differentially expressed NAS can be converted into serotonin by arylacetamide deacetylase [30]. Analyzing the relationship between the DEGs and differential metabolites revealed a close correlation with *HTR2B*. Blood ELISAs revealed elevated 5-HT levels after MMBIT in the NDT, but not in the DT group. This suggested that 5-HT, a potent monoamine neurotransmitter, exerted its biological functions by binding to *HTR2B* in the NDT group. In the central nervous system, 5-HT regulates anxiety, depressive moods, cognition, learning, and attention [31]. Peripheral blood is involved in vascular growth, development, and cardiovascular disease risk [32,33], which 5-HT might alter. Within smooth muscle cells, 5-HT stimulates GPCRs, which results in calcium release from the endoplasmic reticulum and increased intracellular calcium levels. This leads to smooth muscle contraction and narrowing blood vessels, namely vascular stenosis [34], and induces blood vessel vasodilation by activating substance P, 5-HT, adenosine triphosphate (ATP), and M receptors on the endothelial cell surface. This activation leads to the release of endothelium-derived relaxation factors (EDRFs), which ultimately causes vasodilation [35,36]. Nitric acid and nitrogen oxide are structural types of EDRFs that directly impact vascular smooth muscles, stimulate natural vasodilation, and suppress sympathetic vasoconstriction [37]. The effects of 5-HT in blood vessels depend on specific cells and receptors with which it interacts. We found considerably elevated *HTR2B* levels after MMBIT, compared with before intervention in the NDT, but not the DT group. We confirmed that the qPCR findings of blood samples and the transcriptome data concurred. Vasodilation is facilitated by *HTR2B* by influencing endothelial cell behavior, and *HTR2B* also plays a regulatory role in vasodilation, specifically in the coronary and cerebral arteries [36]. The protein expression and localization of *HTR1B* and *HTR2B*, along with dopamine receptors D1 and 2, vary among tumor types, according to a study that screened human telomerase RNA component (HTR) expression in large tumors using functional genomic mRNA analysis. However, endothelial cells express abundant HTR2B protein [38]. 5-HT induces subendocardial left ventricular fibrosis in wild-type mice; this is amplified in mice without *HTR2B* but not in *HTR2A*$^{-}$ or *HTR2A/2B*$^{-/-}$ mice [39]. This suggested that endothelial *HTR2B* regulates coronary vessel diastolic function Furthermore, transcriptomic data revealed obviously increased expression of *GPR82* and *PLPPR4* in the NDT group after than that before intervention. The specialized membrane proteins, GPCRs, comprise seven transmembrane α-helical structures and are ubiquitous in all organ systems [40]. Numerous GPCRs are involved in blood pressure regulation *via* the hypertension polygenic pathway that includes angiotensin receptor 1, which is involved in the renin-angiotensin-aldosterone system [41], and angiotensin receptor type 2 [42]. These receptors influence renal sodium retention and vascular tone [43]. The adrenergic receptor system that affects cardiac contraction and vascular tone is also involved. Some evidence suggests that significant amounts of GPR82 are expressed in the heart and aorta and that this is closely linked to metabolic syndrome. The findings of intracellular *GPR82* agreed with those of *HTR2B*, indicating that the 5-HT signaling pathway initiates a cascade response by regulating *GPR82*. Other genes with a notable upsurge in transcriptome data in the NDT group after therapy included ceramide kinase-like (*CERKL*), which protects cells against oxidative stress, and artesunate (*ARTN*), which hampers oxidative stress and facilitates sustained NO production by endothelial cells. The lipid phosphodiesterase *PLPPR4* expressed only in the neurons in axonal membranes that expand outwards has a distinct function in ending excitatory connections in glutamatergic neurons [44].

Western blots of HUVECs stimulated with Ang II and then incubated with siRNA s revealed a significant decrease in HTR2B, GPR82, and PLPPR4 expression, which was consistent with the transcriptome data. Conversely, S100A2 expression was significantly increased. The protein S100A2 belongs to the S100 family of calcium-binding proteins, and it participates in transforming growth factor (TGF)-β-mediated cell migration and invasion *via* p53 proteins and the TGF-β-induced mitogen-activated protein kinase (MAPK) signaling pathway [45]. Levels of MDA and NO were significantly decreased in the Si-HTR2B, compared with Ang II-induced HUVECs. This suggested that an increased abundance of HTR2B results in a notable increase in MDA and NO. Furthermore, NOS induction that is necessary for NO production was reduced, which aligns with the significantly elevated NOS levels in the HUVECs incubated with Si-HTR2B. Some evidence suggests that myeloid-derived suppressor cells in the brain respond to serotonin by increasing the expression of the serotonin receptor, HTR2B. This, in turn, results in elevated phosphorylated nuclear factor kappa-light-chain-enhancer of activated B cells (pNF-κB) and its associated signaling genes interleukin-6 (*IL-6*), interleukin-1B (*IL-1B*), Cyclooxygenase-2 (*CXCL2*), and Nitric oxide synthase-2 (*NOS-2*) [46]. The intervention with MMBIT resulted in instructions from brain 5-HTP to increase serum 5-HT levels. The transcriptome findings revealed that elevated HTR2B led to increased NO levels.

In summary, the blood-based metabolomes and transcriptomes of patients after MMBIT significantly differed between the DT and NDT groups and were primarily identified in the 5-HT pathway. The expression of HTR2B and 5-HT was significantly higher in the NDT

group after than before the MMBIT intervention. Furthermore, HTR2B suppression led to a notable reduction in HTR2B, GPR82, and PLPPR4 levels. This indicated that MMBIT contributes to the psychological effects of hypertension by regulating 5-HT binding to HTR2B. Furthermore, coexisting 5-HT and HTR2B might serve as a diagnostic marker for managing MMBIT. Controlled studies are required to validate the present findings.

Ethics

All participants provided written informed consent. The Ethics Committee of International Mongolian Medicine Hospital approved this study (Approval No: 2020-014).

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Data availability statement

The data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

Fang Jun: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bao Wenfeng:** Visualization, Validation, Data curation. **Haorile Chagan-Yasutan:** Visualization, Validation, Supervision, Formal analysis, Data curation. **Sarnai Arlud:** Project administration, Formal analysis, Data curation. **Si Qin:** Validation, Supervision, Data curation. **Rihan Wu:** Data curation, Conceptualization. **Nagongbilige He:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

On behalf of all the authors, I declare that no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37113.

Abbreviations

5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
DBP	diastolic blood pressure
ELISA	enzyme-linked immunosorbent assay
FC	fold change
GPCR	G Protein-coupled receptor
HTR2B	5-hydroxytryptamine receptor 2
MDS	malondialdehyde
MMBIT	Mongolian mind–body interactive therapy
NO	nitric oxide
NOS	NO synthetase
PCA	principal component analysis
q-PCR	quantitative polymerase chain reaction
SBP	systolic blood pressure

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