Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Study on the reversal of epileptic drug resistance by tetramethylpyrazine in combination with carbamazepine through modulation of P-Glycoprotein expression in rat brain microvessel endothelial cell

Xia Qian[1], Yuqing Deng[2], Na Zhong[3], Yuxuan Li[3], Junhua Wang[4], Jinhua Yan[5], Wei Liu[6], Jinglei Ye[3], Jingchao Liu[4], Guobei Xiao[4*]

[1] Science and Education Department, The First Hospital of Nanchang, Nanchang 330006, China
[2] Department of Cardiology, The First Hospital of Nanchang, Nanchang 330006, China
[3] Third Clinical Medicine Faculty, Nanchang University, Nanchang 330000, China
[4] Department of Ophthalmology, The First Hospital of Nanchang, Nanchang 330006, China
[5] Department of Rheumatology and Immunology, The First Hospital of Nanchang, Nanchang 330006, China
[6] School of Materials Science and Engineering, Nanchang Hangkong University, Nanchang 330063, China

ARTICLE INFO

Original paper

Article history:
Received: September 28, 2023
Accepted: November 25, 2023
Published: November 15, 2023

Keywords:

tetramethylpyrazine, P-Glycoprotein, carbamazepine, RT-PCR, RNA sequencing

ABSTRACT

The purpose of this study was to detect the changes of P-Glycoprotein (P-GP) expression in rat brain microvessel endothelial cell line RBE4 after the action of Tetramethylpyrazine (TMP) on Carbamazepine (CBZ), so as to clarify the potential mechanism of TMP combined with CBZ against intractable epilepsy drug resistance. The RBE4 cell line was utilized for in vitro analysis. Cells were divided into control, CBZ, and CBZ-TMP group. The expression of P-GP was assessed using Western blot and reverse transcription polymerase chain reaction (RT-PCR). Intracellular concentration of CBZ was measured through high-performance liquid chromatography (HPLC). The differential expression of mRNA was evaluated by RNA sequencing. The intracellular concentration of CBZ in the CBZ-TMP group was significantly higher than that in other groups. The expression of P-GP in the CBZ group was significantly higher than that in the control group, while in the CBZ&TMP group, it was significantly lower than that in the other groups. Comparative analysis also revealed some differentially expressed genes. Compared with the CBZ group, FAM106A, SLC3A2, TENM2, etc. were upregulated most significantly in the CBZ&TMP group. ZBTB10, WDR7, STARD13, etc. were downregulated most significantly. Results suggest that TMP increases the intracellular concentration of CBZ, downregulates the expression of P-GP increased by CBZ, and modulates related cellular metabolism and signaling pathways, thus reversing the drug resistance mechanism of intractable epilepsy, providing a theoretical basis for the combination of traditional Chinese medicine and antiepileptic drugs.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.11.38

Introduction

Epilepsy is a chronic neurological disorder characterized by sudden, repetitive and transient abnormal discharge of brain neurons. It is one of the most prevalent neurological diseases worldwide, with an estimated 50 million people suffering from epilepsy and around 5 million new cases each year (1). Currently, drug therapy remains the main treatment approach for epilepsy, such as first-line antiepileptic drug CBZ. It is effective in controlling abnormal neuronal discharge and relieving epilepsy symptoms by blocking sodium channels (2).

Although most patients can control their condition after treatment, approximately 30% of epilepsy patients will develop intractable epilepsy, a condition that may be caused by drug resistance, which is related to various factors such as drug transporters, drug target sensitivity, epilepsy type, and heredity (3-5). Studying the mechanism of drug resistance to antiepileptic drugs is of great significance for guiding the clinical medication of intractable epilepsy. A key factor in drug resistance is the multidrug transporter P-GP, encoded by the multi-drug resistance (MDR) gene. P-GP facilitates antiepileptic drug excretion from cells, thus reducing intracellular drug concentration and significantly impacting its efficacy and brain concentration (6-9). In addition, Giessmann T et al. demonstrated that the progesterone X receptor (PXR) from the nuclear receptor superfamily has the capability to regulate the expression of P-GP. Specifically, PXR binds to the promoter region of the MDR gene, thereby activating the transcription of P-GP mRNA and resulting in the upregulation of P-GP expression. CBZ, an inducer of PXR, exhibits the ability to effectively enhance the expression level of P-GP by inducing PXR (10-12).

Tetramethylpyrazine (TMP) is one of the key active components found in traditional Chinese medicine Chuanxiong, which possesses various physiological functions, such as antioxidant, anti-inflammatory, anti-apoptotic, regulation of angiogenesis, and neuroprotective properties (13,14). Studies have shown that TMP can reduce the content of intracellular P-GP, thus reversing the multidrug resistance mediated by P-GP, while also increasing the

* Corresponding author. Email: xiaogb1984@163.com

intracellular concentration of other drugs and playing a synergistic role when combined with them (15,16). In this study, we established an RBE4 cell model and used RT-PCR, Western blot, HPLC and RNA sequencing to explore the effect of TMP on the expression of P-GP increased by CBZ and to explain the mechanism of TMP combined with CBZ to reverse drug resistance in intractable epilepsy.

Materials and Methods

Cell culture

RBE4 cells, a type of immortalized rat brain microvessel endothelial cells, were cultured using a mixture of minimum essential medium and Ham's F10 in a 1:1 ratio supplemented with various compounds, including 300 μg/mL of neomycin, 10% FBS, 1 ng/mL of β-FGF, 100 U/mL of penicillin G, 0.25 μg/mL of amphotericin B, 100 μg/mL of streptomycin, 25 mM of sodium bicarbonate, and 25 mM of HEPES. This mixture is referred to as the cell culture medium. The cells were maintained and grown in a humidified atmosphere containing 5% CO_2 at 37°C.

Grouping and drug treatment

TMP and Carbamazepine (CBZ) were procured from MedChemExpress (Monmouth Junction, NJ, USA). The cells were randomly divided into three experimental groups: the control group, the CBZ group, and the CBZ&TMP group. The control group received no medication treatment, while the CBZ group was treated with 200 μM CBZ for 6 hours, 12 hours, Day 1, Day 2, Day 3, Day 4, Day 5, Day 6and Day 7. The CBZ&TMP group was simultaneously treated with 200 μM CBZ and 300 μg/mL TMP, with the same treatment durations as the CBZ group.

Quantification of P-GP mRNA by RNA isolation and qPCR

The RNA was isolated by chloroform-isopropanol-ethanol protocol from tissue and cells using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA). SYBR Green for quantitative polymerase chain reaction (qPCR) was used to perform real-time reverse transcription (RT) qPCR using 1 g RNA input. Primers were synthesized as follows: rat P-GP forward primer: 5'-AGCGACAGCAGTCTGGAGGA-3', and reverse primer: 5'-GACGTCATCTGTGAGCCGGG-3'; GAPDH forward: 5′-CAAGAAGGTGGTGAAGCAG-3′ and GAPDH reverse: 5'-CAAAGGTGGAAGAATGGGAG-3'. mRNA quantification was normalized to the housekeeping gene GAPDH and represented as fold change (2–ΔΔCt).

Quantification of P-GP by Western Blot

Cells lysates were prepared by digesting the tissues in 1X lysis buffer, protease and phosphatase inhibitor and PMSF (Sangon Biotech, Shanghai, China) in a Qiagen Tissue Lyser for 8 min at 50 rpm at 4°C, rocked for 1h at 4°C and sonicated. For 10% SDS-PAGE electrophoresis, 20 g of each lysate was processed using the PierceTM BCA protein assay (Thermo Fisher, Waltham, MA, USA). SDS PAGE gels were transferred to PVDF (Millipore, Billerica, MA, USA) and blocked with 5%BSA (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 25°C. At a concentration of 1:1000, primary anti-P-GP antibodies (Abcam, ab129450, Cambridge, MA, USA) were incubated at 4°C rocking. A secondary HRP antibody was incubated for one hour at room temperature under rocking conditions. High-signature ECL Western Blot Substrate (Tanon, Shanghai, China) was used to develop the blots.

Quantification of CBZ by high-performance liquid chromatography

The separation procedure was carried out at a temperature of 22°C using a μBondapak C-18 column. The mobile phase, which consisted of aqueous 30 mM potassium phosphate buffer (with pH adjusted to 3.7 with 5% phosphoric acid) and acetonitrile in a ratio of 65:35, was delivered at a flow rate of 1.2 mL/min. Detection was performed using a Waters 486 detector, with monitoring carried out at a wavelength of 270 nm.

Analysis of differentially expressed genes by mRNA sequencing

For the library construction process, a total of 1 μg of RNA was extracted from the specified RBE4 cell lines using TRIZOL reagent. Ribo-Zero rRNA Removal Kits (Illumina, San Diego, CA, USA) were then used according to the manufacturer's instructions to remove the rRNAs from the RNA samples. Subsequently, the rRNA-depleted RNAs were used to construct RNA sequencing libraries with the TruSeq Stranded Total RNA Library Prep Kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol. Each extracted RNA sample was used for the preparation of the RNA sequencing library, cDNA synthesis, and PCR amplification, with a selection of approximately 150 bp PCR amplicons size of products. The constructed RNA-Seq libraries were quality-controlled and quantified using the BioAnalyzer 2100 system (Agilent Technologies, Inc., Santa Clara, CA, USA). Finally, the library sequencing process was performed on an Illumina Hiseq instrument with 150 bp paired-end reads.

Statistical analysis

Statistical analysis in this study was conducted using SPSS 24.0 software (IBM, located in Armonk, NY, USA). To assess distinctions between the two groups, the Student's t-test was employed. For comparisons involving multiple groups, the One-way ANOVA test was utilized, followed by a Post Hoc Test (Least Significant Difference). A significance level of $p<0.05$ was adopted to determine statistical significance.

Results

Tetramethylpyrazine elevated the intracellular level of CBZ in RBE4 cells

To understand the effect of TMP on the intracellular level of CBZ in RBE4 cells, we detected the intracellular concentration of CBZ in RBE4 cells with or without TMP treatment. After HPLC detection, we found that in the CBZ&TMP group, the intracellular concentration of CBZ was significantly higher than in the CBZ group after 24 hours and remained constant after the fifth day (Figure 1). This result suggests that TMP elevates the intracellular level of CBZ in RBE4 cells.

Tetramethylpyrazine reversed the CBZ-elevated expression of P-GP in RBE4 cells

To evaluate the effect of TMP and CBZ on the expression level of P-GP in the RBE4 cells, we treated the RBE4

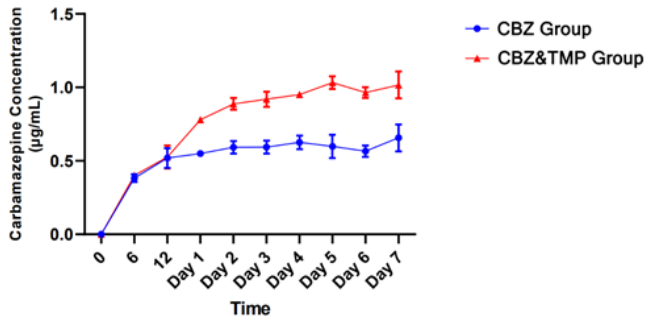

Figure 1. The time-dependence effects of Tetramethylpyrazine on the intracellular CBZ concentration in RBE4 cells in the indicated times (0 h, 6 h, 12 h, day 1, day 2, day 3, day 4, day 5, day 6 and day 7) were analyzed by HPLC.

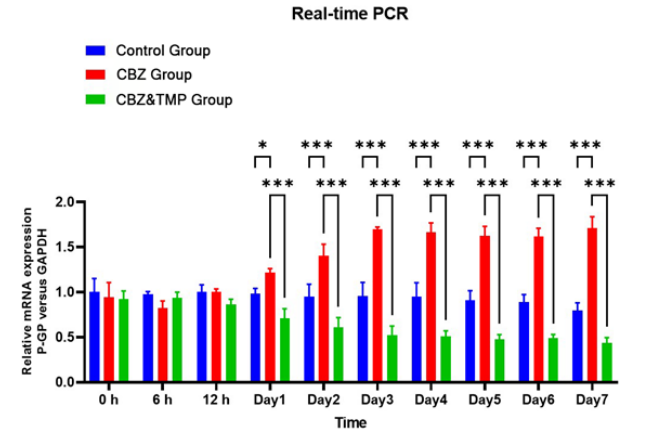

Figure 2. The time-dependence effects of CBZ with or without Tetramethylpyrazine on the mRNA expression level of P-GP in RBE4 cells were analyzed by Real-time PCR. *P<0.05, **P<0.01, and ***P<0.001 versus indicated group.

cells with or without TMP and CBZ. The result showed that its level in the CBZ treatment group gradually increased as the treatment time extended and maintained a stable level after 72 hours. In contrast, the mRNA expression level of P-GP in the RBE4 cells of the CBZ&TMP group gradually decreased as the treatment time extended and maintained a stable level after 72 hours (Figure 2). Consistently, the protein expression level of P-GP in the RBE4 cells of the CBZ group gradually increased as the treatment time extended and maintained a stable level after 72 hours. In contrast, the protein expression level of P-GP in the RBE4 cells of the CBZ&TMP group gradually decreased as the treatment time extended and maintained a stable level after 72 hours (Figure 3). These results suggested that TMP significantly reversed the CBZ-elevated expression of P-GP in RBE4 cells.

TMP combined with CBZ can modulate cellular metabolism and signaling pathways

To gain a better understanding of the impact of TMP and CBZ on RBE4 cell mRNA expression, we performed mRNA sequencing on the control group, the CBZ group, and the CBZ&TMP group. In comparison to the control group, the CBZ group exhibited upregulation of 1,284 genes and downregulation of 743 genes (Figure 4). The ten genes that displayed the most significant upregulation were PIK3CG, GOLGA8S, LMX1B, CD200, POU4F2, ARHGEF16, NKD2, SLC18A1, DLX5, and MLIP. Conversely, the ten genes that exhibited the most

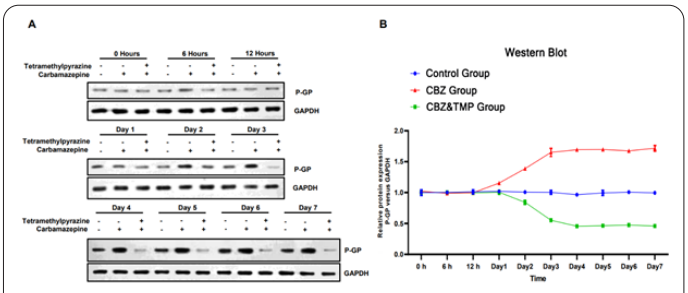

Figure 3. The time dependence effects of CBZ with or without Tetramethylpyrazine on the protein expression level of P-GP in RBE4 cells. (A) Western blot analysis of P-GP protein level in RBE4 cells at different times in RBE4. (B) Line chart showing the effects of CBZ with or without Tetramethylpyrazine on the protein P-GP levels in RBE4 cells in the indicated times (0 h, 6 h, 12 h, day 1, day 2, day 3, day 4, day 5, day 6 and day 7). All of the value P is below 0.001 between each group.

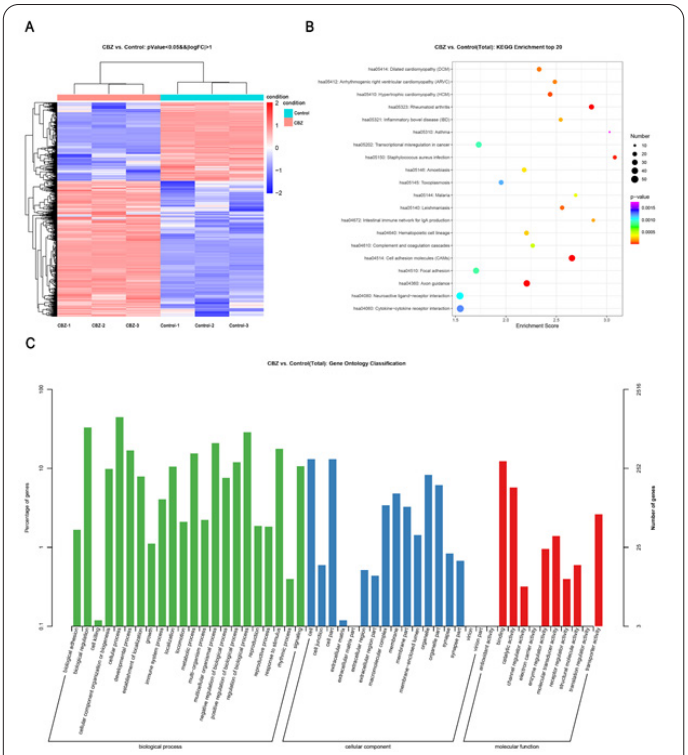

Figure 4. CBZ vs. Control: Differential expression analysis and functional analysis of mRNAs of RNA-seq. (A) Hierarchical clustering analysis (heatmap) for differently expressed genes pValue<0.05&|logFC|>1Gene. (B) The KEGG analysis (q-value < 0.05) of the top 20 differently expressed genes. (C) Gene ontology (GO) term enrichment analysis.

significant downregulation were SLITRK1, GYPE, PPP4R3C, OCA2, PRDM13, MAOB, TRO, CSMD1, COX7B2, and GPX7 (Table 1). In comparison to the CBZ group, the CBZ&TMP group showed upregulation of 699 genes and downregulation of 645 genes (Figure 5). The ten genes that displayed the most significant upregulation were FAM106A, SLC3A2, TENM2, MUC4, RAD51, VIP, ZNF516, PPP2R5A, DNAH11, and COMMD8. Conversely, the ten genes that exhibited the most significant downregulation were ZBTB10, WDR7, STARD13, GABRA2, RAB4A, SCRG1, TRIM39, ZNF155, HOXD3, and PSMD6 (Table 1). The thermogram analysis revealed notable differences between the CBZ group and the CBZ&TMP group.

To assess the biological functionality of differen-

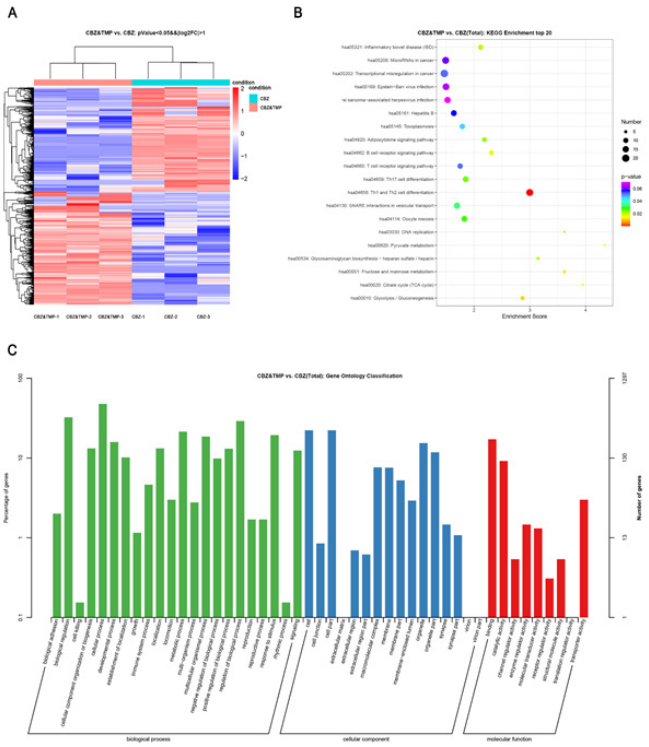

Figure 5. CBZ&TMP vs. CBZ: Differential expression analysis and functional analysis of mRNAs of RNA-seq. (A) Hierarchical clustering analysis (heatmap) for differently expressed genes pValue<0.05&|logFC|>1. (B) The KEGG analysis (q-value < 0.05) of the top 20 differently expressed genes. (C) Gene ontology(GO)term enrichment analysis.

tially expressed mRNAs between the CBZ group and the CBZ&TMP group, we conducted GO and KEGG enrichment analyses on the mRNA data. In GO enrichment analysis, the significant difference of mRNA in the CBZ&TMP group is mainly related to binding, catalytic activity, and transporter activity in molecular function. In the cellular component, it is mainly related to the cell, cell part, organelle, and organelle part. In biological processes, it is mainly related to cellular component organization or biogenesis, biological regulation, regulation of biological processes, metabolic processes, response to stimuli, and so on. The KEGG enrichment analysis was conducted with a condition of q-value < 0.05. Significant differences in mRNA enrichment were observed in several metabolic pathways, including pyruvate metabolism, citrate cycle (TCA cycle), fructose and mannose metabolism, DNA replication, glycosaminoglycan biosynthesis-heparan sulfate/heparin, Th1 and Th2 cell differentiation, and glycolysis/gluconeogenesis in the CBZ&TMP group.

Discussion

TMP is a traditional Chinese medicine active ingredient that exhibits a universally effective therapeutic effect for multiple systemic diseases. Studies have indicated its extensive application in clinical cardiovascular and cerebrovascular diseases, liver injury diseases, and cancer in China, with positive evaluations of its efficacy (17-20). Tao et al.'s study revealed that TMP can reduce epileptic seizures and improve efficacy when used in combination with conventional antiepileptic drugs (21). Its mechanism of action may involve multiple targets, pathways, and bidirectional regulation, making it a drug with immense research potential.

This study represents the first investigation into the potential mechanisms underlying the combined use of TMP and CBZ in intractable epilepsy and its impact on drug resistance. The experimental drug employed in this study was CBZ, and the findings revealed that TMP is capable of augmenting the concentration of CBZ within RBE4 cells, thus providing further evidence for the synergistic effect between TMP and antiepileptic drugs. This synergistic effect may be attributed to the reduction of P-GP concentration by TMP, which weakens the role of P-GP protein in promoting drug efflux from cells and finally leads to an increase in CBZ concentration in cells. Further investigations revealed that TMP can reverse the upregulation of P-GP mRNA and P-GP induced by CBZ, maintaining their expression at sustained low levels, similar to the inhibitory effect of P-GP inhibitors. Yinxu Zhang, et al.'s study also demonstrated that TMP can inhibit P-GP ATPase activity and suppress P-GP expression (22). Furthermore, enrichment analysis of mRNA from different groups revealed significant enrichment of several metabolic pathways, such as acetate metabolism, fructose and mannose metabolism, and the TCA cycle, upon the addition of TMP. This suggests that the reversal of CBZ resistance by TMP may be associated with cellular energy metabolism pathways. The reversing effect of TMP may be associated with its antioxidant activity, CenJuan, Hao Hong, Yafang Zhang, et al.'s study indicated that reactive oxygen species (ROS) in cells can induce overexpression of P-GP, whereas TMP can significantly inhibit the generation of ROS (23-25).

This study also has some limitations. Firstly, we conducted in vitro experiments using REB4 cells, which cannot fully replicate the complex microenvironment of tissues and organs. Therefore, further in vivo experiments and clinical observations are needed to complement the results obtained from in vitro cell experiments in order to obtain more accurate and comprehensive research conclusions. Secondly, in RNA sequencing and enrichment ana-

Table 1. The top ten most upregulated genes and the top ten most downregulated genes in each group of mRNA sequencing data.

Group	Regulation	Gene Id
CBZ group vs. control group	Up	PIK3CG, GOLGA8S, LMX1B, CD200, POU4F2, ARHGEF16, NKD2, SLC18A1, DLX5, MLIP
	Down	SLITRK1, GYPE, PPP4R3C, OCA2, PRDM13, MAOB, TRO, CSMD1, COX7B2, GPX7
CBZ&TMP group vs. CBZ group	Up	FAM106A, SLC3A2, TENM2, MUC4, RAD51, VIP, ZNF516, PPP2R5A, DNAH11, COMMD8
	Down	ZBTB10, WDR7, STARD13, GABRA2, RAB4A, SCRG1, TRIM39, ZNF155, HOXD3, PSMD6

lysis, we did not deeply analyze the regulatory changes of various genes and signaling pathways. In future research, we can further explain the detailed drug resistance mechanism of TMP combined with CBZ in regulating cell metabolism and signaling pathways from the gene level.

Our study has confirmed that the combination of TMP and CBZ can reduce the expression of P-GP, thereby reversing drug resistance in epilepsy. This provides a solid experimental foundation and theoretical basis for the clinical application of traditional Chinese medicine in combination with antiepileptic drugs for the treatment of intractable epilepsy, offering a new approach to the use of antiepileptic drug combinations and the management of intractable epilepsy. Furthermore, TMPs have demonstrated neuroprotective and antidepressant effects in treating comorbidities of epilepsy, such as improving cognitive impairment and regulating anxiety and depression levels, thus presenting broad application prospects (26). However, there is still a need for sufficient clinical research data on the clinical dosage, efficacy, and prognosis of TMP, which necessitates further investigation and analysis in future studies.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Funding

This work was supported by the Nanchang Science and Technology Support Project (Grant No. Hongkezi [2019] 258); Guobei Xiao was supported by the China Primary Health Care Foundation under Grant No. MTP2022D028; Wei Liu was supported by the Natural Science Foundation of Jiangxi Province under Grant No. 20212BAB214040.

Data availability statement (DAS)

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

References

1. Zhang D, Cui X, Zheng J, et al. A Retrospective Study of Parietal Lobe Epilepsy: Functional Anatomy and Surgical Treatment. Altern Ther Health M 2022; 28(6): 138-143.
2. Loscher W, Potschka H, Sisodiya SM, Vezzani A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. Pharmacol Rev 2020; 72(3): 606-638.
3. Naimo GD, Guarnaccia M, Sprovieri T, et al. A Systems Biology Approach for Personalized Medicine in Refractory Epilepsy. Int J Mol Sci 2019; 20(15): 3717.
4. Zierath D, Mizuno S, Barker-Haliski M. Frontline Sodium Channel-Blocking Antiseizure Medicine Use Promotes Future Onset of Drug-Resistant Chronic Seizures. Int J Mol Sci 2023; 24(5): 4848.
5. Tan M, Kavurmaci M. Complementary And Alternative Medicine Use In Turkish Patients With Epilepsy. Altern Ther Health M 2021; 27(4): 19-22.
6. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat Rev Cancer 2018; 18(7): 452-464.
7. Stefan K, Schmitt SM, Wiese M. 9-Deazapurines as Broad-Spectrum Inhibitors of the ABC Transport Proteins P-Glycoprotein, Multidrug Resistance-Associated Protein 1, and Breast Cancer Resistance Protein. J Med Chem 2017; 60(21): 8758-8780.
8. Zybina A, Anshakova A, Malinovskaya J, et al. Nanoparticle-based delivery of carbamazepine: A promising approach for the treatment of refractory epilepsy. Int J Pharmaceut 2018; 547(1-2): 10-23.
9. Giessmann T, May K, Modess C, et al. Carbamazepine regulates intestinal P-glycoprotein and multidrug resistance protein MRP2 and influences disposition of talinolol in humans. Clin Pharmacol Ther 2004; 76(3): 192-200.
10. Ke XJ, Cheng YF, Yu N, Di Q. Effects of carbamazepine on the P-gp and CYP3A expression correlated with PXR or NF-kappaB activity in the bEnd.3 cells. Neurosci Lett 2019; 690: 48-55.
11. Elmeliegy M, Vourvahis M, Guo C, Wang DD. Effect of P-glycoprotein (P-gp) Inducers on Exposure of P-gp Substrates: Review of Clinical Drug-Drug Interaction Studies. Clin Pharmacokinet 2020; 59(6): 699-714.
12. Lin J, Wang Q, Zhou S, Xu S, Yao K. Tetramethylpyrazine: A review on its mechanisms and functions. Biomed Pharmacother 2022; 150: 113005.
13. Liu Y, Yang G, Cui W, Zhang Y, Liang X. Regulatory mechanisms of tetramethylpyrazine on central nervous system diseases: A review. Front Pharmacol 2022; 13: 948600.
14. Yang S, Wu S, Dai W, et al. Tetramethylpyrazine: A Review of Its Antitumor Potential and Mechanisms. Front Pharmacol 2021; 12: 764331.
15. Wang S, Lei T, Zhang M. The Reversal Effect and Its Mechanisms of Tetramethylpyrazine on Multidrug Resistance in Human Bladder Cancer. Plos One 2016; 11(7): e157759.
16. Guo M, Liu Y, Shi D. Cardiovascular Actions and Therapeutic Potential of Tetramethylpyrazine (Active Component Isolated from Rhizoma Chuanxiong): Roles and Mechanisms. Biomed Res Int 2016; 2016: 2430329.
17. Gao HJ, Liu PF, Li PW, et al. Ligustrazine monomer against cerebral ischemia/reperfusion injury. Neural Regen Res 2015; 10(5): 832-840.
18. Qian W, Xiong X, Fang Z, Lu H, Wang Z. Protective effect of tetramethylpyrazine on myocardial ischemia-reperfusion injury. Evid-Based Compl Alt 2014; 2014: 107501.
19. Zhao Y, Liu Y, Chen K. Mechanisms and Clinical Application of Tetramethylpyrazine (an Interesting Natural Compound Isolated from Ligusticum Wallichii): Current Status and Perspective. Oxid Med Cell Longev 2016; 2016: 2124638.
20. Tao F, Cai Y, Deng C, Chen Z, Shen Y, Sun H. A narrative review on traditional Chinese medicine prescriptions and bioactive components in epilepsy treatment. Ann Transl Med 2023; 11(2): 129.
21. Zhang Y, Ma C, He L, et al. Tetramethylpyrazine Protects Endothelial Injury and Antithrombosis via Antioxidant and Antiapoptosis in HUVECs and Zebrafish. Oxid Med Cell Longev 2022; 2022: 2232365.
22. Cen J, Zhang L, Liu F, Zhang F, Ji BS. Long-Term Alteration of Reactive Oxygen Species Led to Multidrug Resistance in MCF-7 Cells. Oxid Med Cell Longev 2016; 2016: 7053451.
23. Hong H, Lu Y, Ji ZN, Liu GQ. Up-regulation of P-glycoprotein expression by glutathione depletion-induced oxidative stress in rat brain microvessel endothelial cells. J Neurochem 2006; 98(5): 1465-1473.
24. Zhang Y, Liu X, Zuo T, Liu Y, Zhang JH. Tetramethylpyrazine reverses multidrug resistance in breast cancer cells through regulating the expression and function of P-glycoprotein. Med Oncol 2012; 29(2): 534-538.
25. Lee B, Shim I, Lee H, Hahm DH. Tetramethylpyrazine reverses anxiety-like behaviors in a rat model of post-traumatic stress disorder. Korean J Physiol Pha 2018; 22(5): 525-538.
26. Cui H, Xu Z, Qu C. Tetramethylpyrazine ameliorates isoflurane-induced cognitive dysfunction by inhibiting neuroinflammation

via miR-150 in rats. Exp Ther Med 2020; 20(4): 3878-3887.