Life Sciences 121 (2015) 46–56

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

iTRAQ-based proteomic analysis of tetramethylpyrazine inhibition on lipopolysaccharide-induced microglial activation

Qiang-Hong Pu, Jun-Lin He, Ming-Jun Wu, Jia-Jia Li, Zhu Yang, Ying-Xiong Wang, Chao Yu *

Institute of Life Science and School of Public Health, Chongqing Medical University, Chongqing 400016, PR China

ARTICLE INFO

Article history:
Received 26 May 2014
Accepted 14 November 2014
Available online 2 December 2014

Keywords:
Tetramethylpyrazine
Microglia
Neurodegenerative diseases
ITRAQ
Proteomics

ABSTRACT

Aims: Neurodegenerative diseases are the leading cause of morbidity and mortality worldwide. Several studies have shown that tetramethylpyrazine (TMP) is an effective therapy for neurodegenerative diseases and that it acts by inhibiting the activation of microglial cells in response to inflammatory stimuli. However, the molecular mechanisms underlying the action of TMP remain unknown.
Main methods: Proteomic analysis was used to generate novel insights into the mechanism by which TMP inhibits microglial activation, and western blotting was used to validate candidate proteins.
Key findings: To identify candidate proteins affected by TMP in lipopolysaccharide-activated microglia, we performed proteomic analysis using iTRAQ labelling coupled with LC TRIPLE-TOF, and we identified 5187 unique proteins. Among these, 266 proteins were differentially expressed and considered putative candidate proteins. Protein annotation revealed that the differentially expressed proteins, such as inducible nitric oxide synthase (iNOS) and ERO1-like protein (ERO1L), might be involved in reducing cellular oxidation in response to stress. Ingenuity pathway analysis revealed that the differentially expressed proteins were involved in a variety of signalling pathways, including liver X receptor/retinoid X receptor (LXR/RXR) activation and the production of nitric oxide and reactive oxygen species in macrophages. Furthermore, one of the differentially expressed protein candidates detected by iTRAQ, iNOS, was confirmed by western blotting.
Significance: Our data suggest that iTRAQ technology is an effective tool to study the mechanism by which TMP inhibits activated microglia. TMP decreased the expression of LXR/RXR-mediated iNOS, which reduced microglial activation in response to inflammatory stimuli.

Introduction

Neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, are common disorders in the elderly population, affecting approximately 22 million worldwide, and they are associated with substantial morbidity and mortality. However, the underlying pathogenesis of neurodegenerative diseases remains unclear. Studies indicate that the characteristic neuronal degeneration involving in neurodegenerative diseases is mediated by oxidative stress and neuroinflammation and that microglia, the resident macrophages in the central nervous system, play a key role in these processes [2,7,19]. Microglia are the major regulators that maintain homeostasis in response to infection, injury or immunological stimuli in the central nervous system [1,9,16]. Normally, activated microglia prevent the initiation and progression of neurodegenerative diseases through the release of trophic and anti-inflammatory cytokines [12,22]. However, in response to irresolvable inflammatory stimuli, over-activated microglia promote selective neuronal loss and the acceleration of neurodegenerative diseases via excess release of pro-inflammatory cytokines and neurotoxic mediators, including tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), reactive oxygen species (ROS), and nitric oxide (NO) [1,10,24,25].

In recent years, efforts have been made to discover potential therapeutic compounds for neurodegenerative diseases. Tetramethylpyrazine (TMP), also known as ligustrazine, is a biologically active compound isolated from *Ligusticum wallichii*, and it is widely used to treat several disorders including asthma, myocardial injury and rhinitis in China [26]. Accumulating evidence suggests that TMP may be a potential therapeutic candidate for neurodegenerative diseases [17,20,21]. *In vivo* and *in vitro* studies demonstrated that TMP could directly block neuronal cell loss and brain oxidative damage, as well as attenuate impairment of learning and memory by promoting the recovery of antioxidative functions such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) ([20,21,23,27]). Activated microglia significantly contribute to the development of neurodegenerative disorders. However, few studies have focused on the TMP-mediated inhibitory effect on the overproduction of inflammatory and oxidative mediators in activated microglia. Our preliminary studies indicate that TMP suppresses the release of NO from activated microglia by reducing inducible nitric oxide synthase (iNOS) expression

* Corresponding author at: Box 174#, Institute of Life Sciences, Chongqing Medical University, No. 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, PR China. Tel.: +86 23 68485589; fax: +86 23 68486294.
E-mail address: yuchaom@163.com (C. Yu).

http://dx.doi.org/10.1016/j.lfs.2014.11.016
0024-3205/

Table 1
Differentially up-regulated (>2-fold) and down-regulated (<0.5-fold) proteins identified by iTRAQ after TMP, LPS and TMP-LPS treatment.

Accession number	Gene symbols	Protein names	Peptides	TMP vs ctrl		LPS vs ctrl		TMP-LPS vs ctrl	
				Fold-change	p-Value	Fold-change	p-Value	Fold-change	p-Value
Proteins deregulated between TMP, LPS and TMP-LPS treatments[a]									
Down/down/down									
Q91ZH2	PCNA	Proliferating cell nuclear antigen	24	0.5	4.1E−02	0.3	2.9E−02	0.4	9.0E−03
P02535	KRT10	Keratin, type I cytoskeletal 10	12	0.3	7.1E−03	0.4	1.9E−02	0.1	9.9E−04
Up/up/up									
Q3UBY9	P4HB	Putative uncharacterised protein	73	2.5	9.0E−03	4.5	9.2E−10	2.4	5.8E−04
Q4FE56	USP9X	Ubiquitin carboxyl-terminal hydrolase	19	3.0	1.0E−03	2.3	3.9E−02	2.5	2.5E−03
F7AC58	PIEZO1	Piezo-type mechanosensitive ion channel component 1 (Fragment)	8	2.4	2.6E−02	2.7	1.2E−02	2.5	2.6E−02
E9PVX6	MKI67	Protein Mki67	4	2.8	4.3E−02	4.6	3.9E−02	6.4	3.6E−02
Proteins deregulated between TMP and LPS treatments[a]									
Down/down									
Q8VCT0	LDLR	Low density lipoprotein receptor	6	0.4	2.1E−02	0.3	3.6E−02		
Up/up									
Q9CT10	RANBP3	Ran-binding protein 3	10	2.1	3.7E−02	3.5	1.1E−03		
Q3TCX3	KIAA0907	UPF0469 protein KIAA0907	4	6.8	1.8E−02	6.6	1.1E−02		
F8WIE1	MAN2C1	Alpha-mannosidase 2C1	3	15.3	3.2E−02	11.2	3.9E−02		
Q80TM9	NISCH	Isoform 3 of Nischarin	6	5.2	4.6E−02	6.4	3.7E−02		
Proteins deregulated between TMP and TMP-LPS treatments[a]									
Up/up									
Q9ESZ8	GTF2I	Isoform 5 of General transcription factor II-I	23	2.3	1.5E−02			2.0	2.1E−02
Q8K295	LRPAP1	Lrpap1 protein (Fragment)	24	2.2	2.7E−02			2.0	2.0E−02
Q99ME9	GTPBP4	Nucleolar GTP-binding protein 1	8	2.4	3.2E−02			2.7	2.5E−02
Proteins deregulated between LPS and TMP-LPS treatments[a]									
Down/down									
Q64737	GART	Trifunctional purine biosynthetic protein adenosine-3	37			0.4	1.2E−05	0.5	1.2E−03
Proteins deregulated at the TMP treatment[a]									
Down									
Q8CD23	NCL	Putative uncharacterised protein	73	0.5	1.5E−02				
Q9D0I9	RARS	Arginine–tRNA ligase, cytoplasmic	35	0.4	1.7E−02				
Q08943	SSRP1	FACT complex subunit SSRP1	18	0.5	1.9E−02				
Q3URU4	MRE11A	Meiotic recombination 11 homologue A (S. cerevisiae), isoform CRA_c	14	0.3	4.2E−02				
Q3UVN5	NSFL1C	Putative uncharacterised protein	13	0.4	2.7E−02				
Q3UE11		Putative uncharacterised protein	14	0.2	3.1E−02				
Q99JR1	SFXN1	Sideroflexin-1	13	0.3	1.5E−02				
Q9ESU7	SLC1A5	Neutral amino acid transporter ASCT2	12	0.3	4.6E−02				
Q8BPX9	SLC15A3	Solute carrier family 15 member 3	10	0.4	1.4E−02				
Q4FJY5	PTGR1	Ltb4dh protein	8	0.2	2.5E−02				
Q8K2E5	BUB1B	Budding uninhibited by benzimidazoles 1 homologue, beta (S. cerevisiae)	8	0.4	1.4E−02				
Q3UCV8	FAM105B	Ubiquitin thioesterase otulin	3	0.2	1.6E−02				
Up									
F8VQC7	KTN1	Kinectin	21	2.0	2.4E−02				
Q07417	ACADS	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	17	2.2	4.5E−02				
Q3V3N6	COPS2	Putative uncharacterised protein	13	2.6	1.1E−02				
G5E8I8	CHERP	Calcium homeostasis endoplasmic reticulum protein	12	2.9	7.7E−03				
P63242	EIF5A	Eukaryotic translation initiation factor 5A-1	20	2.1	1.7E−02				
Q6ZPJ3	UBE2O	Ubiquitin-conjugating enzyme E2 O	11	2.2	1.4E−02				
Q3UP61	DLG1	Putative uncharacterised protein	10	2.2	4.1E−02				
Q91W39	NCOA5	Nuclear receptor coactivator 5	8	2.3	8.3E−03				
A2AWT6	UBTF	Nucleolar transcription factor 1	9	2.6	4.0E−02				
Q3UD86	MOV10	Putative uncharacterised protein	6	2.2	3.1E−02				
Q8K215	LYRM4	LYR motif-containing protein 4	4	2.0	3.6E−02				
E9Q8V6	DENND4A	Protein Dennd4a	8	2.4	1.9E−02				
Q8VE99	CCDC115	Coiled-coil domain-containing protein 115	3	3.2	4.2E−02				
B2RRX2	PPP3CA	Serine/threonine-protein phosphatase	4	4.0	4.8E−02				
Q8BME2	NDUFA12	Putative uncharacterised protein	3	3.4	4.0E−02				
Q9EPR4	SLC23A2	Solute carrier family 23 member 2	4	2.7	2.8E−03				

(continued on next page)