Potential pharmacological treatment of fragile X syndrome during adulthood

Zhao-Hui XU, Ming-Gao ZHAO

Department of Pharmacology, School of Pharmacy, Fourth Military Medical University, Xi'an 710032, China

Abstract: Fragile X syndrome (FXS) is the most common form of inherited mental retardation, characterized by moderate-to-severe mental retardation, attention deficits, and hyperactivity. This disease results from the expansion of a trinucleotide repeat (CGG) within the X-linked fragile X mental retardation 1 (*FMR1*) gene, which leads to the lack of the product of the *FMR1* gene—fragile X mental retardation protein. Many mental disorders such as FXS and Rett syndrome are thought to originate during early developmental period, but recent findings have suggested the involvement of the processes in the adult nervous system. Here we outline our recent studies and initial clinical trials that may provide an approach to treat FXS in the adulthood.

Keywords: fragile X syndrome; central nervous system; dopamine; clinical trials

Fragile X syndrome (FXS) is the most common form of inherited mental retardation, characterized by moderate-to-severe mental retardation, attention deficits, and anxiety. This disease results from the expansion of a trinucleotide repeat (CGG) within the X-linked fragile X mental retardation 1 (*FMR1*) gene^[1-4], thus the product of the *FMR1* gene—fragile X mental retardation protein (FMRP) is not expressed^[1, 5, 6]. FMRP is an mRNA-binding protein that is associated with polyribosomes and is thought to be involved in the translational efficiency and/or trafficking of certain mRNAs^[7, 8].

A myriad of studies have defined spatial and associative learning deficits in *FMR1* knockout (*Fmr1*^{-/-}) mice. While mild spatial learning deficits have been reported, the role of *FMR1* in fear conditioning remains elusive, since some studies have reported intact contextual fear memory^[9], while others report deficits in both auditory and contextual fear conditioning^[10]. FXS is often accompanied by neuropsychiatric

disorders such as hyperactivity, attention disorders, and autism^[11-13]. Deficits in activity-dependent synaptic plasticity have also been observed in piriform cortices and hippocampal CA1 region in *Fmr1*^{-/-} mice^[14-17]. Long-term potentiation (LTP), a type of long lasting synaptic plasticity, is believed to be involved in learning and memory^[18, 19].

Dysregulated mGluR signaling and altered protein synthesis may play central roles in the pathophysiology of FXS, including associated behavioral and cognitive impairment^[12, 20]. In *Fmr1*^{-/-} mice model, decreasing group I mGluR signaling is corrected with a plethora of FXS-related phenotypes^[21]. Acute pharmacological suppression of mGluR signaling in a mouse model of FXS during adulthood could prevent auditory seizures and ameliorate the abnormalities in exploratory behavior, suggesting that alteration in mGluR signaling contributes to FXS-related neurological phenotypes in this model of disorder^[20].

Researchers recently have constructed an adeno-associated virus-based viral vector that expresses the major central nervous system (CNS) isoform of FMRP. They found that hippocampal synaptic PP-LFS-induced LTD was restored to WT level in *Fmr1*^{-/-} mice that had received hippocampal injection of vector. These results indicate that expression of

Corresponding author: Ming-Gao ZHAO
Tel: 86-29-84774552; Fax: 86-29-84774552
E-mail: minggao@fmmu.edu.cn
Article ID: 1673-7067(2009)05-0296-05
CLC number: R596
Document code: A
Received date: 2009-09-09

the major CNS isoform of FMRP alone is sufficient to rescue this phenotype, and post-developmental protein replacement may have the potential to improve cognitive function in FXS^[22].

Our previous studies have demonstrated a defect in trace fear memory in *Fmr1*^{-/-} mice, while locomotion, anxiety and pain sensitivity are intact^[16]. Whole-cell patch-clamp recordings in the anterior cingulate cortex (ACC) showed that LTP was completely abolished in *Fmr1*^{-/-} mice. A similar decrease in LTP was also found in the lateral amygdala, another structure implicated in fear memory. No significant changes were found in basal synaptic transmission. This suggests that synaptic plasticity in the ACC and amygdala in *Fmr1*^{-/-} mice play an important role in the expression of behavioral phenotypes similar to the symptoms of FXS. In the same study, we also demonstrate that trace fear conditioning is impaired in *Fmr1*^{-/-} mice, indicating that the *Fmr1* gene is important in attention-based associative learning. Trace fear conditioned learning requires the intact medial prefrontal cortex, ACC and hippocampus^[17, 23-25].

Consistent with the defect in trace fear conditioning in *Fmr1*^{-/-} mice, patients with FXS also exhibit abnormal activation patterns in the ventromedial prefrontal cortex, ACC and hippocampus^[26]. Trace fear conditioning requires the subject to sustain attention during the trace interval^[24, 27]. Since children with Fragile X exhibit severe behavioral problems of inattention^[28, 29] and many are diagnosed with attention-deficit hyperactivity disorder (ADHD)^[30], the use of trace fear conditioning may be more sensitive to mild learning deficits in *Fmr1*^{-/-} mice compared to traditional measures of conditioned fear memory.

Dopamine (DA) in the prefrontal cortex (PFC) plays a critical role in cognitive functions and neuropsychiatric pathology^[31-35]. It is well known that DA functions in its target cells through 5 subtypes of DA receptors (D1-5)^[36-38]. Studies have been carried out by numerous groups to investigate the cellular mechanism of DA modulation in PFC neurons^[33, 34, 39-43]. Our previous study has revealed that FMRP contributes to dopamine modulation of AMPA GluR1 receptor synaptic insertion and dopaminergic facilitation of LTP^[44]. By using biochemical methods, we found that the surface expression and phosphorylation of AMPA GluR1 re-

ceptors at the cAMP-dependent protein kinase (PKA) phosphorylation site (Ser845) induced by stimulated D1 receptors was attenuated in *Fmr1*^{-/-} PFC neurons. Consistent with the results from cultured neurons, DA-mediated facilitation of LTP was absent in cingulate region of PFC slices of adult *Fmr1*^{-/-} mice. In the cultured PFC neurons of *Fmr1*^{-/-} mice, cell surface expression and phosphorylation of the GluR1 glutamate receptors, normally mediated by the D1 receptors, were reduced. Normally, D1 receptors are coupled to Gs protein, which stimulates the production of an intermediary signalling molecule called cyclic AMP, by activating the enzyme adenylate cyclase. In *Fmr1*^{-/-} mice, the ability of the D1-receptor agonist to raise cAMP level in PFC neurons is attenuated. Kinase enzymes known as GRKs can phosphorylate D1 receptors at several residues, diminishing the receptors' ability to interact with Gs. In PFC neurons from *Fmr1*^{-/-} mice, the phosphorylation levels of D1 receptors are abnormally high. We hypothesize that FMRP normally interacts with GRK2 and prevents its activity. Hence, in the absence of FMRP in *Fmr1*^{-/-} mice, GRK2 is no longer regulated and thus becomes overactive. Moreover, GRK2 seems to be free to move to the cell membrane, where it phosphorylates D1 receptors and disrupts their signaling^[44]. Pharmacological inhibition of GRK2 can rescue D1 receptor signaling and its downstream effect on AMPA GluR1 receptors. The hyperphosphorylation of D1 receptor at serine sites and the GRK2 increase in cell membrane in both PFC and striatum contribute to the impairment of D1 receptor signaling in *Fmr1*^{-/-} mice. On the other hand, FMRP expression in *Fmr1*^{-/-} neurons or pharmacological inhibition of GRK2 could rescue D1 receptor signaling and its downstream effects on GluR1 receptors in *Fmr1*^{-/-} neurons^[44]. Our observation that deregulation of GRK2 activity in the absence of FMRP leads to impaired dopamine signaling is a reminder that we still have much to learn on the molecular, cognitive and behavioural manifestations of this disorder^[45].

Mouse and fly models of FXS serve as an important starting point for studying the underlying neurobiological mechanism and the development of possible target for treatment. Recent studies indicate that neurological impairment of neurodevelopmental disorders may be reversible in

the mature nervous system, even if the treatment is started after cessation of development^[20]. Findings from animal models and preliminary clinical trials support this concept in FXS, the single gene neurodevelopmental disorder. As we know, dysregulated mGluR signaling and altered protein synthesis may play central roles in the pathophysiology of FXS, including the associated behavioral and cognitive impairment^[12, 20]. However, acute pharmacological suppression of mGluR signaling during adulthood by mGluR antagonist MPEP and lithium could prevent abnormal behavioral impairment^[46, 47].

The overactivation of the mGluR pathway is believed to cause many symptoms of fragile X, including hyperactivity and anxiety. Drugs developed to target the mGluR pathway have exerted excellent effects in FXS animal models, but they are not yet available for humans. Recently, fenobam, a drug previously used for anxiety, has been identified as a potent and specific mGluR blocker and now is evaluated as effective in the treatment of FXS^[48]. A double-blind placebo-controlled clinical trial of fenobam in children with FXS will be initiated as soon as possible based on FDA regulations for drug development. The potential beneficial clinical effects seen in the pilot trial support the further study of the compound in adults with FXS.

Lithium has been shown to inhibit (partially block) the mGluR pathway and reverse the phenotypes in mutant fly and *Fmr1*^{-/-} mice. Its therapeutic effects in bipolar disorder have been ascribed to the inhibition of GSK3^[49, 50]. Lithium can directly inhibit GSK3, which is amplified *in vivo* by a subsequent increase in the inhibitory serine phosphorylation of GSK3^[51]. Depending on this theory, several selective small molecules of ATP competitive inhibitors of GSK3 have also been developed^[52-54]. A pilot add-on trial has been conducted to evaluate the safety and efficacy of lithium in humans with FXS^[47], and results from this study are consistent with those in mouse and fly models of FXS, which suggests that lithium is well-tolerated and provides functional benefits in FXS, possibly by modifying the underlying neural defect.

A Phase II, 4-week randomized, double-blind, placebo-controlled clinical trial has been conducted to evaluate the safety and efficacy of the ampakine compound CX516 as a

potential drug against the underlying disorder in FXS. However, results showed no significant improvement in memory, the primary outcome measure, or in the secondary measures of language, attention/executive function, behavior, and overall functioning in CX516-treated subjects, compared to the placebo. Failure in the potency of CX516 in other studies suggests that the dosage may be inadequate for therapeutic effect, thus it remains unclear whether modulation of AMPA-mediated neurotransmission is a viable therapeutic strategy for the treatment of FXS^[55].

Taken together, animal model studies and initial clinical trials serve as an important starting point for studying the underlying neurobiological mechanism and the development of possible treatment for FXS. It is indicated that pharmacological treatment leads to functional improvement, even when the treatment is started in adulthood. Initial clinical trial findings can help us further evaluate the potential therapeutics for the treatment of this single-gene neurodevelopmental disorder.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (No. 30670684, 30770686).

References:

- [1] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, *et al.* Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; 65 (5): 905-914.
- [2] Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell* 1994; 77 (1): 33-39.
- [3] Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, *et al.* Translational suppression by trinucleotide repeat expansion at FMR1. *Science* 1995; 268 (5211): 731-734.
- [4] Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, *et al.* Length of uninterrupted CGG repeats determines instability in the FMR1 gene. *Nat Genet* 1994; 8 (1): 88-94.
- [5] Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, *et al.* Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 1991; 252 (5010):

- 1097-1102.
- [6] Kooy RF. Of mice and the fragile X syndrome. *Trends Genet* 2003; 19 (3): 148-154.
- [7] Jin P, Warren ST. New insights into fragile X syndrome: from molecules to neurobehaviors. *Trends Biochem Sci* 2003; 28 (3): 152-158.
- [8] Willemsen R, Oostra BA, Bassell GJ, Dichtenberg J. The fragile X syndrome: from molecular genetics to neurobiology. *Ment Retard Dev Disabil Res Rev* 2004; 10 (1): 60-67.
- [9] Van Dam D, D'Hooge R, Hauben E, Reyniers E, Gantois I, Bakker CE, *et al.* Spatial learning, contextual fear conditioning and conditioned emotional response in *Fmr1* knockout mice. *Behav Brain Res* 2000; 117 (1-2): 127-136.
- [10] Paradee W, Melikian HE, Rasmussen DL, Kenneson A, Conn PJ, Warren ST. Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. *Neurosci* 1999; 94 (1): 185-192.
- [11] Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci* 2005; 6 (5): 376-387.
- [12] Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci* 2004; 27 (7): 370-377.
- [13] Grossman AW, Aldridge GM, Weiler IJ, Greenough WT. Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. *J Neurosci* 2006; 26 (27): 7151-7155.
- [14] Larson J, Jessen RE, Kim D, Fine AK, du Hoffmann J. Age-dependent and selective impairment of long-term potentiation in the anterior piriform cortex of mice lacking the fragile X mental retardation protein. *J Neurosci* 2005; 25 (41): 9460-9469.
- [15] Lauterborn JC, Rex CS, Kramar E, Chen LY, Pandeyarajan V, Lynch G, *et al.* Brain-derived neurotrophic factor rescues synaptic plasticity in a mouse model of fragile X syndrome. *J Neurosci* 2007; 27 (40): 10685-10694.
- [16] Zhao MG, Toyoda H, Ko SW, Ding HK, Wu LJ, Zhuo M. Deficits in trace fear memory and long-term potentiation in a mouse model for fragile X syndrome. *J Neurosci* 2005; 25 (32): 7385-7392.
- [17] Huerta PT, Sun LD, Wilson MA, Tonegawa S. Formation of temporal memory requires NMDA receptors within CA1 pyramidal neurons. *Neuron* 2000; 25 (2): 473-480.
- [18] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993; 361 (6407): 31-39.
- [19] Malenka RC, Nicoll RA. NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci* 1993; 16 (12): 521-527.
- [20] Ehninger D, Silva AJ. Genetics and neuropsychiatric disorders: treatment during adulthood. *Nat Med* 2009; 15 (8): 849-850.
- [21] Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, *et al.* Correction of fragile X syndrome in mice. *Neuron* 2007; 56 (6): 955-962.
- [22] Zeier Z, Kumar A, Bodhinathan K, Feller JA, Foster TC, Bloom DC. Fragile X mental retardation protein replacement restores hippocampal synaptic function in a mouse model of fragile X syndrome. *Gene Ther* 2009.
- [23] Runyan JD, Moore AN, Dash PK. A role for prefrontal cortex in memory storage for trace fear conditioning. *J Neurosci* 2004; 24 (6): 1288-1295.
- [24] Han CJ, O'Tuathaigh CM, van Trigt L, Quinn JJ, Fanselow MS, Mongeau R, *et al.* Trace but not delay fear conditioning requires attention and the anterior cingulate cortex. *Proc Natl Acad Sci U S A* 2003; 100 (22): 13087-13092.
- [25] Blank T, Nijholt I, Kye MJ, Radulovic J, Spiess J. Small-conductance, Ca²⁺-activated K⁺ channel SK3 generates age-related memory and LTP deficits. *Nat Neurosci* 2003; 6 (9): 911-912.
- [26] Menon V, Leroux J, White CD, Reiss AL. Frontostriatal deficits in fragile X syndrome: relation to FMR1 gene expression. *Proc Natl Acad Sci U S A* 2004; 101 (10): 3615-3620.
- [27] Clark RE, Squire LR. Classical conditioning and brain systems: the role of awareness. *Science* 1998; 280 (5360): 77-81.
- [28] Baumgardner TL, Reiss AL, Freund LS, Abrams MT. Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Pediatrics* 1995; 95 (5): 744-752.
- [29] Fryns JP, Jacobs J, Kleczkowska A, van den Berghe H. The psychological profile of the fragile X syndrome. *Clin Genet* 1984; 25 (2): 131-134.
- [30] Cornish K, Sudhalter V, Turk J. Attention and language in fragile X. *Ment Retard Dev Disabil Res Rev* 2004; 10 (1): 11-16.
- [31] Matsuda Y, Marzo A, Otani S. The presence of background dopamine signal converts long-term synaptic depression to potentiation in rat prefrontal cortex. *J Neurosci* 2006; 26 (18): 4803-4810.
- [32] Snyder SH. Dopamine receptor excess and mouse madness. *Neuron* 2006; 49 (4): 484-485.
- [33] Surmeier DJ. Dopamine and working memory mechanisms in prefrontal cortex. *J Physiol* 2007; 581 (Pt 3): 885.
- [34] Huang YY, Simpson E, Kellendonk C, Kandel ER. Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proc Natl Acad Sci U S A* 2004; 101 (9): 3236-3241.
- [35] Carlsson A. A half-century of neurotransmitter research: impact on neurology and psychiatry. *Nobel lecture. Biosci Rep* 2001; 21 (6): 691-710.
- [36] West AR, Grace AA. Opposite influences of endogenous dopam-

- ine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. *J Neurosci* 2002; 22 (1): 294-304.
- [37] Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* 1995; 376 (6541): 572-575.
- [38] Sun X, Zhao Y, Wolf ME. Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci* 2005; 25 (32): 7342-7351.
- [39] Tseng KY, O'Donnell P. Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J Neurosci* 2004; 24 (22): 5131-5139.
- [40] Chen G, Greengard P, Yan Z. Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. *Proc Natl Acad Sci U S A* 2004; 101 (8): 2596-2600.
- [41] Li N, Gao Z, Luo D, Tang X, Chen D, Hu Y. Selenium level in the environment and the population of Zhoukoudian area, Beijing, China. *Sci Total Environ* 2007; 381 (1-3): 105-111.
- [42] Trantham-Davidson H, Neely LC, Lavin A, Seamans JK. Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. *J Neurosci* 2004; 24 (47): 10652-10659.
- [43] Wang H, Wu LJ, Kim SS, Lee FJ, Gong B, Toyoda H, *et al.* FMRP acts as a key messenger for dopamine modulation in the forebrain. *Neuron* 2008; 59 (4): 634-647.
- [44] Weinshenker D, Warren ST. Neuroscience: fragile dopamine. *Nature* 2008; 455 (7213): 607-608.
- [45] de Vrij FM, Levenga J, van der Linde HC, Koekkoek SK, De Zeeuw CI, Nelson DL, *et al.* Rescue of behavioral phenotype and neuronal protrusion morphology in *Fmr1* KO mice. *Neurobiol Dis* 2008; 31 (1): 127-132.
- [46] Berry-Kravis E, Sumis A, Hervey C, Nelson M, Porges SW, Weng N, *et al.* Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. *J Dev Behav Pediatr* 2008; 29 (4): 293-302.
- [47] Berry-Kravis E, Hessler D, Coffey S, Hervey C, Schneider A, Yuhass J, *et al.* A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet* 2009; 46 (4): 266-271.
- [48] Jope RS. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol Sci* 2003; 24 (9): 441-443.
- [49] Phiel CJ, Klein PS. Molecular targets of lithium action. *Annu Rev Pharmacol Toxicol* 2001; 41: 789-813.
- [50] De Sarno P, Li X, Jope RS. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. *Neuropharmacology* 2002; 43 (7): 1158-1164.
- [51] Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci* 2003; 116 (Pt 7): 1175-1186.
- [52] Kozikowski AP, Gaisina IN, Petukhov PA, Sridhar J, King LT, Blond SY, *et al.* Highly potent and specific GSK-3beta inhibitors that block tau phosphorylation and decrease alpha-synuclein protein expression in a cellular model of Parkinson's disease. *ChemMedChem* 2006; 1 (2): 256-266.
- [53] Martinez A, Alonso M, Castro A, Perez C, Moreno FJ. First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3beta) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J Med Chem* 2002; 45 (6): 1292-1299.
- [54] Berry-Kravis E, Krause SE, Block SS, Guter S, Wu J, Leurgans S, *et al.* Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: a controlled trial. *J Child Adolesc Psychopharmacol* 2006; 16 (5): 525-540.

成年脆性 X 综合症的潜在药物治疗

许朝晖, 招明高

第四军医大学药理学系药理学教研室, 西安 710033

摘要: 脆性 X 综合症是临床最为常见的遗传性智力发育迟滞综合症, 其特征性表现包括中度至严重的智力发育障碍、注意力降低、多动等。发病原因是 X- 基因连锁的脆性基因(*FMR1*)CGG 序列过度重复表达, 导致 *FMR1* 基因沉默, 其表达产物 FMRP 缺失。脆性 X 综合症与 Ratt 综合症等被认为起源于早期的发育障碍, 但最近的研究表明, 成年动物中枢神经系统功能异常也参与疾病的病理发展。本文对最近的研究结果加以综述, 并对潜在的成年脆性 X 综合症治疗的相关临床试验进行探讨。

关键词: 脆性 X 综合症; 中枢神经系统; 多巴胺; 临床试验