ISSN 2220-3206 (online)

World Journal of *Psychiatry*

World J Psychiatr 2020 July 19; 10(7): 150-174

Published by Baishideng Publishing Group Inc

World Journal of Psychiatry

Contents

Monthly Volume 10 Number 7 July 19, 2020

ORIGINAL ARTICLE

Basic Study

SYSTEMATIC REVIEWS

World Journal of Psychiatry

Monthly Volume 10 Number 7 July 19, 2020

Contents

NAME OF JOURNAL
World Journal of Psychiatry

ISSN
ISSN 2220-3206 (online)

LAUNCH DATE
December 31, 2011

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Rajesh R Tampi

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2220-3206/editorialboard.htm

PUBLICATION DATE
July 19, 2020

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Psychiatry

Submit a Manuscript: https://www.f6publishing.com

World J Psychiatr 2020 July 19; 10(7): 150-161

DOI: 10.5498/wjp.v10.i7.150

ISSN 2220-3206 (online)

ORIGINAL ARTICLE

Basic Study

Comparison of three administration modes for establishing a zebrafish seizure model induced by N-Methyl-D-aspartic acid

Xin-Yi Long, Shuang Wang, Zhi-Wen Luo, Xu Zhang, Hong Xu

ORCID number: Xin-Yi Long 0000-0003-0265-9645; Wang Shuang 0002-0002-6053-3998; Zhi-Wen Luo 0000-0002-0524-9951; Xu Zhang 0000-0001-8916-5626; Hong Xu 0000-0003-4938-0203.

Author contributions: Long XY, Wang S, Luo ZW and Zhang X conceived the study; Wang S, Long XY and Luo ZW performed the experiments; Long XY, Wang S and Luo ZW analyzed the data; Luo ZW, Wang S and Long XY drafted the manuscript; All authors read and approved the final version of the manuscript; Xu H provided the idea and funding; Long XY and Wang S contributed equally.

Supported by the National Natural Science Foundation of China, No. 81760216, No. 81160144 and No. 31171044.

Institutional animal care and use committee statement: The study was approved by the Ethical Review Committee of Nanchang University.

Conflict-of-interest statement: All other authors have nothing to disclose.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The

Xin-Yi Long, Shuang Wang, Queen Mary School of Nanchang University, Nanchang 330006, Jiangxi Province, China

Zhi-Wen Luo, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200433, China

Xu Zhang, Affiliated Eye Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Hong Xu, Institute of Life Science, Nanchang University, Nanchang 330031, Jiangxi Province, China

Corresponding author: Hong Xu, PhD, Professor, Institute of Life Science, Nanchang University, Xuefu Avenue 1299, Nanchang 330031, Jiangxi Province, China.
xuhong@ncu.edu.cn

Abstract

BACKGROUND

Epilepsy is a complex neurological disorder characterized by recurrent, unprovoked seizures resulting from the sudden abnormal discharge of brain neurons. It leads to transient brain dysfunction, manifested by abnormal physical movements and consciousness. It can occur at any age, affecting approximately 65 million worldwide, one third of which are still estimated to suffer from refractory seizures. There is an urgent need for further establishment of seizure models in animals, which provides an approach to model epilepsy and could be used to identify novel anti-epileptic therapeutics in the future.

AIM

To compare three administration modes for establishing a seizure model caused by N-Methyl-D-aspartic acid (NMDA) in zebrafish.

METHODS

Three administration routes of NMDA, including immersion, intravitreal injection and intraperitoneal injection, were compared with regard to their effects on inducing seizure-like behaviors in adult zebrafish. We evaluated neurotoxicity by observing behavioral changes in zebrafish and graded those behaviors with a seizure score. In addition, the protective effects of MK-801 (Dizocilpine) and

authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Manuscript source: Invited manuscript

Received: December 31, 2019
Peer-review started: December 31, 2019
First decision: April 3, 2020
Revised: April 25, 2020
Accepted: May 26, 2020
Article in press: May 26, 2020
Published online: July 19, 2020

P-Reviewer: Ng QX
S-Editor: Dou Y
L-Editor: Webster JR
E-Editor: Wu YXJ

natural active constituent resveratrol against NMDA-induced alterations were studied.

RESULTS
The three NMDA-administration methods triggered different patterns of the epileptic process in adult zebrafish. Seizure scores were increased after increasing NMDA concentration regardless of the mode of administration. However, the curve of immersion continuously rose to a high plateau (after 50 min), while the curves of intravitreal injection and intraperitoneal injection showed a spike in the early stage (10-20 min) followed by a steady decrease in seizure scores. Furthermore, pretreatment with resveratrol and MK-801 significantly delayed seizure onset time and lowered seizure scores.

CONCLUSION
By comparing the three methods of administration, intravitreal injection of NMDA was the most suitable for establishing an acute epileptic model in zebrafish. Thus, intraperitoneal injection in zebrafish can be applied to simulate diseases such as epilepsy. In addition, NMDA immersion may be an appropriate method to induce persistent seizures. Moreover, MK-801 and resveratrol showed strong anti-epileptic effects; thus, both of them may be clinically valuable treatments for epilepsy.

Key words: Seizure; Zebrafish; N-Methyl-D-aspartic acid; Administration modes; Resveratrol; MK-801

Core tip: This is the first study to systematically compare the three main administration modes to establish a seizure model in zebrafish. A newly developed zebrafish model with acute and sustained experimental epileptic behavior enables us to study and identify potential mechanisms and screen anti-epileptic drugs. Direct administration of N-Methyl-D-aspartic acid stimulates abnormal excitations of brain nerve cells to simulate epileptic seizures. This study demonstrated that intravitreal injection can be used to establish an acute epilepsy model and immersion can be used as a persistent epilepsy model. The protective effects of resveratrol and MK-801 on the epileptic process were also confirmed, which may have clinical application value.

Citation: Long XY, Wang S, Luo ZW, Zhang X, Xu H. Comparison of three administration modes for establishing a zebrafish seizure model induced by N-Methyl-D-aspartic acid. *World J Psychiatr* 2020; 10(7): 150-161
URL: https://www.wjgnet.com/2220-3206/full/v10/i7/150.htm
DOI: https://dx.doi.org/10.5498/wjp.v10.i7.150

INTRODUCTION

Epilepsy is a chronic brain disorder caused by abnormal, excessive and synchronous neuronal activities in the brain. The clinical manifestations are characterized by paroxysmal, transient, repetitive and stereotyped. The location of abnormal discharge neurons and the range of abnormal discharge spread are different, leading to different forms of seizure, manifested as sensory, motor, conscious, mental, behavioral, autonomic dysfunction or a combination of multiple dysfunctions[1]. According to the World Health Organization report, there are many causes of epilepsy, such as stroke, brain trauma, and central nervous system infection[2]. Although it is generally believed that about two-thirds of epilepsy is idiopathic, most of which are now considered to be hereditary[1]. Epilepsy also has various psychiatric complications such as depression, anxiety and cognitive defects[3,4]. It has been reported that peroxisome proliferator-activated receptor γ and mutations in genes involved in GABA-mediated inhibitory neurotransmission are highly associated with the pathogenesis of epilepsy[1,5]. Moreover, it is widely accepted that glutamate overstimulation of the N-Methyl-D-aspartic acid (NMDA) receptor is an important pathogenesis of epilepsy, which leads

to continuous internal flow of calcium ions and excessive excitement of the hippocampal networks[6,7]. The molecular mechanism of epilepsy is still not fully understood, and thus there is a lack of effective clinical treatment[8,9]. Therefore, building relevant preclinical models is imperative for therapeutics screening in this disease.

NMDA is an amino acid derivative that exists naturally in the animal body. It is an analog of L-glutamate, an important excitatory neurotransmitter in the mammalian central nervous system. It has been used to model a series of neurodegenerative diseases such as epilepsy, glaucoma, Alzheimer's disease, Parkinson's disease and Huntington's disease[10-15]. NMDA-induced cellular excitotoxicity may be the consequence of overstimulating NMDA receptors at high concentrations of NMDA, causing a massive calcium influx. The overproduction of nucleases, proteinases, lipase, free radicals as well as the activation of nitric oxide pathway ultimately then give rise to cell death[16,17].

As a vertebrate model, zebrafish have received a great deal of attention in the field of developmental biology and genetics over the last decade as a cost-efficient and relevant alternative for human disease modeling and large-scale drug screening[18]. There are many reasons for its popularity, for example, zebrafish share high genetic, cellular and organ homologies to humans over the evolutionary process[19]. Besides their homology, zebrafish are much easier to breed than other types of experimental animals due to ease of handling and fast reproduction rate. These advantages indicate that zebrafish has outstanding value in preclinical drug screening[20,21]. Furthermore, its central nervous system is structurally similar to that of mammals. Some signaling systems such as serotonin energy and GABAergic neurotransmission are also highly similar[18,22]. When it comes to studying brain disease, these aspects have always been the advantages of zebrafish. Establishing a reliable zebrafish epilepsy model not only contributes to a better understanding of the molecular pathology of zebrafish seizures, but may also be conducive to screen drugs that protect the brain from seizure damage. Both of these directions will contribute to better clinical treatment of epilepsy.

The principal methods of administration used in the present study were immersion, intravitreal injection and intraperitoneal injection. Some studies have demonstrated that drugs can be absorbed directly from the water environment through the skin of zebrafish[23,24], while intraperitoneal injection of drugs can induce epileptic behaviors in zebrafish[25,26]. Ouabain was injected into the eyeballs of zebrafish through the vitreous cavity, resulting in nerve cell damage[27,28]. Although there are other alternative modeling methods, such as intraperitoneal perfusion, they were not adopted in this study.

MK-801 is a non-competitive antagonist of the NMDA receptor and can directly prevent NMDA-induced excitatory toxicity. Therefore, it was used as a positive control. Resveratrol is a biologically active constituent extracted from many plants. As previously reported, it has a multitude of health benefits including the ability to prolong life and prevent certain diseases such as heart disease, autoimmune diseases, metabolic disorders[29], inflammation[30], neurodegeneration[31,32], and epilepsy[33,34]. In addition, it also plays an active role in retinal degeneration models[35]. However, it is not clear whether the three modes of NMDA administration cause changes in epileptic behavior, and whether resveratrol or MK-801 can protect against this brain disorder. This study aims to investigate these two key aspects to further understand the establishment of seizure models in zebrafish and lay the foundation for drug screening and treatment development.

MATERIALS AND METHODS

Animals

The study was approved by the Ethical Review Committee of Nanchang University (Nanchang, Jiangxi Province, China). The China Zebrafish Resource Center (Wuhan, Hubei Province, China) provided adult male and female wild-type zebrafish (*Danio rerio*, AB strain). All adult zebrafish were raised in a temperature-controlled (28°C) zebrafish breeding system (Thmorgan Biotechnology Corp., Ltd. Beijing, China) and all zebrafish were propagated in a cycle of 14 h light/10 h dark in the experiment, and they were fed with brine shrimp twice daily (Wudi, Shandong Province, China).

Treatments

NMDA (M3262, Sigma, United States) was dissolved in phosphate buffered saline (PBS) to prepare solutions for different modeling methods (immersion: 300 and 500

μmol/L; intraperitoneal injection: 8 and 16 mg/kg; intravitreal injection: 0.1 and 0.5 mol/L). MK-801 (M107, Sigma, United States) was soluble in 500 mL/L ethanol (intraperitoneal injection: 3 mg/kg, intravitreal injection: 0.05 mol/L). Resveratrol (R5010, Sigma, United States) was dissolved in 1000 mL/L ethanol and kept in the dark during storage and during the whole experiment (40 mg/L). Distilled water was used to dissolve MS-222 (A5040, Sigma, United States) (0.2 g/L). The timeline of drug delivery is shown in Figure 1.

Study design

Immersion: Before NMDA treatment, zebrafish were soaked in resveratrol solution for 1 d in advance. Resveratrol was dissolved in 10 μL of 1000 mL/L ethanol, and then mixed with 100 mL of distilled water for 1 h until completely dissolved. During resveratrol treatment, the reaction tank was completely covered to avoid photodegradation of the compound. Then adult zebrafish (n = 6 in each group) were immersed in 500 μmol/L NMDA solution for 1 h (the solution was prepared at 28°C) and seizure-like behaviors were observed for 1 h. Another two groups of zebrafish, 12 in total without resveratrol immersion were separately placed into the other two 2 L tanks, respectively, filled with 300 μmol/L and 500 μmol/L concentration of NMDA for 1 h to record behavioral changes. Zebrafish in the MK-801 group were intraperitoneally injected with 10 μL MK-801 1 h before 500 μmol/L NMDA immersion. The remaining zebrafish were set as the control group, and except for drug treatment, the rest of the process was the same as the experimental groups.

Intravitreal injection: Zebrafish were anesthetized with MS-222 before intravitreal injection. According to previous research methods, the volume of the vitreous cavity measured by digital caliper is approximately 200-500 nL[36]. In the preliminary experiment to determine the appropriate amount of injection, we found that 100 nL PBS did not cause any retinal damage or behavior changes in zebrafish. The freshly prepared 100 nL of 0.1 and 0.5 mol/L NMDA solution was then aspirated with an acupuncture needle (0.20 mm) and inserted through a small incision between the vitreous body and the retina and delivered into the right eyes of zebrafish. The syringe pumps (HARVARD, C-14171) helped to deliver the appropriate amount of NMDA. The drug treatment time was 1 h. For the resveratrol + NMDA group, these zebrafish were treated with resveratrol for 1 d before 0.5 mol/L NMDA injection. In the 0.5 mol/L NMDA + MK-801 group, 100 nL MK-801 was injected intravitreally. 1× PBS injection was performed in the same manner to the control group. After the injection, all the fish were kept out for at least 1 min to allow for drug absorption and then returned to their normal living environment to record their behaviors.

Intraperitoneal injection: Each zebrafish was weighed before the injection and then adult zebrafish were anesthetized with MS-222 solution. NMDA 8 mg/kg and 16 mg/kg (the doses used here were chosen by comparing those used in rodents) was carefully injected into the abdomen in the operating area when the zebrafish temporarily lost body control. For the resveratrol + NMDA group, these zebrafish were treated with resveratrol for 1 d before the NMDA injection. Next, 10 μL MK-801 was injected intraperitoneally in the NMDA+MK-801 group. The remaining fish were injected with 1× PBS and set as the control group. Their 1 h behaviors were recorded *via* camera.

Seizure score

Following intravitreal and intraperitoneal injection, the zebrafish were individually placed into 2 L tanks after NMDA treatment; for immersion, the fish were directly placed in 2 L NMDA solution. We manipulated a specific camera to monitor the behavior of each fish to determine its seizure score. The behaviors of all zebrafish were photographed for 60 min to assess the degree of epilepsy. The seizure score was quantified by the following criteria: 1 point, immobility and hyperventilation; 2 points, whirlpool swimming; 3 points, rapid movement from right to left; 4 points, abnormal and spastic muscle contraction; 5 points, rapid clonic convulsion of the whole body; 6 points, submergence and spasm for several minutes; 7 points, death[25].

Statistical analysis

Statistical analysis was performed based on records to calculate time points and duration of abnormal behavior in zebrafish. The experimental data were expressed as mean ± SE. All data were analyzed by t-test using GraphPad PRISM 7.00. ANOVA was then performed to assess the differences in seizure and latency between the experimental groups, with $P < 0.05$ considered statistically significant.

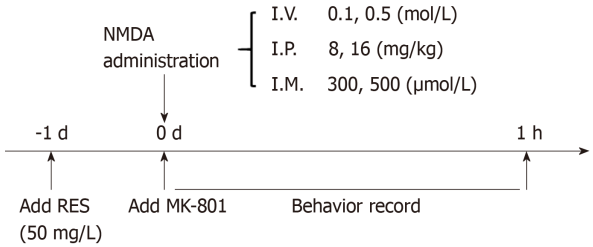

Figure 1 Experimental protocols. Three administration modes were used in this study, including intravitreal injection, intraperitoneal injection and immersion. All resveratrol treatments were carried out 1 d before N-Methyl-D-aspartic acid (NMDA) treatment. Behavior records began immediately after NMDA and MK-801 treatment, which lasted for 1 h. NMDA: N-Methyl-D-aspartic acid; I.V.: Intravitreal injection; I.P.: Intraperitoneal injection; I.M.: Immersion.

RESULTS

Intravitreal injection

Prior to NMDA treatment, zebrafish were immersed in 40 mg/L resveratrol for 1 d. Zebrafish behavior was then observed and recorded for 60 min after intravitreal injection of NMDA and graded by the seizure score. NMDA treatment caused a seizure-like syndrome characterized by rapid movement, jumping, swimming in circles, and an intense response to stimulation. As shown in Figure 2A, the overall curve presented a trend of an intial rise and then a decrease, and the highest seizure score was approximately up to 5. The mean seizure score in the high-dose (0.5 mol/L) NMDA treatment group was significantly higher than that in the low-dose (0.1 mol/L) NMDA treatment group (4 *vs* 2, $P < 0.0001$), while co-injection of 50 mmol/L MK-801 with 100 nL 0.5 mol/L NMDA decreased the score to lower than 2. The shortest seizure onset time in zebrafish given intravitreal injection was about 3 min in the high-dose NMDA group. Additionally, the seizure onset time following high-dose NMDA treatment was significantly prolonged by MK-801 (> 10 min), to a level even lower than that of low-dose (0.1 mol/L) NMDA treatment (Figure 2B). Pretreatment with resveratrol also lowered the seizure score from approximately 4 to 1 and significantly delayed seizure onset from 3 to around 7 min ($P = 0.0024$) (Figure 2A and B). These behavioral changes indicate that intravitreal injection of NMDA leads to seizure-like behavior in zebrafish within a short time that can be significantly prevented by resveratrol pretreatment, consistent with prior studies showing the anti-epileptic effects of resveratrol.

Intraperitoneal injection

For resveratrol pretreatment, we immersed zebrafish in 50 mg/kg resveratrol for 1 d and then performed intraperitoneal injection of NMDA. Zebrafish behaviors were recorded for 60 min, and were similar to those following intravitreal injection of NMDA. According to the analysis of seizure score, the overall curve first increased within 30 min and then decreased following intraperitoneal injection. The degree of epilepsy in zebrafish injected with high dose NMDA was significantly higher than that in zebrafish injected with low dose NMDA. The seizure score in the high-dose (16 mg/kg) NMDA group was 4-5, while the low-dose (8 mg/kg) group had a score of around 2 (Figure 3A). The same trend in seizure onset time was identified: 16 mg/kg NMDA-treated zebrafish had a seizure onset time of less than 10 min while the 8 mg/kg NMDA-treated group had a seizure onset time closer to 20 min (Figure 3B). On the other hand, zebrafish treated intraperitoneally with 10 μL 3 mg/kg MK-801 + high-dose NMDA, had a significantly reduced seizure score at all time points analyzed ($P < 0.001$) (Figure 3A) as well as a very significantly delayed seizure onset time (from approximately 6 min to over 30 min) ($P < 0.001$) (Figure 3B). Resveratrol pretreatment also had a highly significant effect in lowering the seizure score from approximately 5 to 2 min (Figure 3A) and delayed seizure onset time from around 4 min to about 8 min ($P = 0.0024$) (Figure 3B). These data indicate that seizures can be more intensely induced in zebrafish by intraperitoneal injection of NMDA as well as intravitreal injection and that MK-801 and resveratrol have anti-epileptic effects in this model system.

Immersion

Zebrafish in one experimental group were placed into 2 L tanks with 40 mg/L resveratrol for 1 d before NMDA immersion. By recording zebrafish behaviors over 60 min, we found that different groups almost showed an increasing trend. Seizure scores

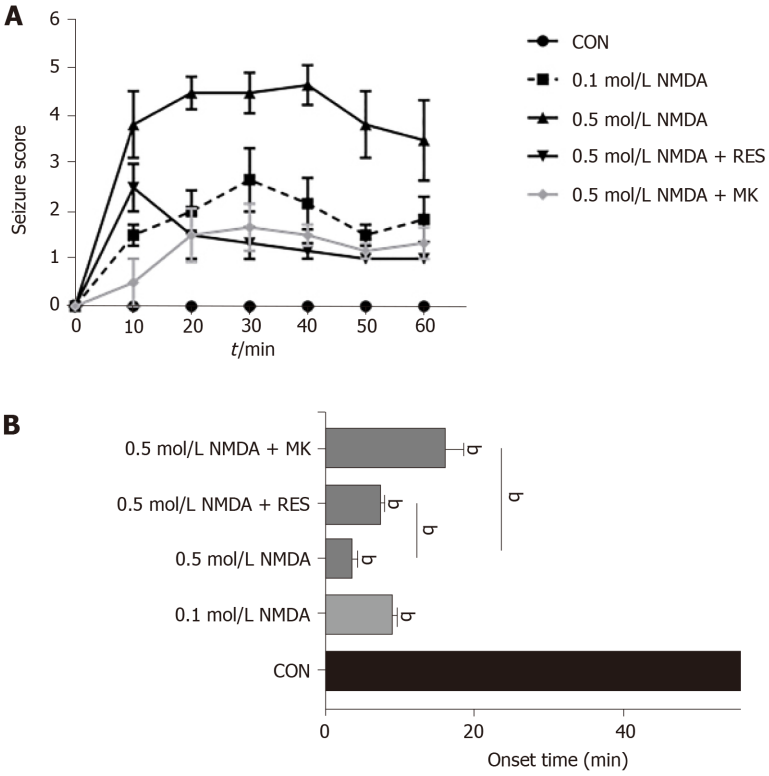

Figure 2 Intravitreal injection of N-Methyl-D-aspartic acid causes acute seizure-like behavior in zebrafish. Seizure activity and onset scores following N-Methyl-D-aspartic acid (NMDA)-intravitreal injection (0.1 mol/L and 0.5 mol/L, 50 mg/L resveratrol + 0.5 mol/L NMDA, and 50 mmol/L MK-801 + 0.5 mol/L NMDA). A: The mean seizure scores (± SE) for each treatment group plotted against time after NMDA injection; B: The latency of seizure onset after NMDA injection. Bars represent mean ± SE of the time lag to the onset of the first convulsion in each group. Data were analyzed using one-way ANOVA and *t*-test for the different groups (n = 6) ($^bP < 0.01$ *vs* control). NMDA: N-Methyl-D-aspartic acid; RES: Resveratrol; MK: MK-801.

continued to rise steadily within 0-20 min and 30-50 min. In addition, seizure scores following high-dose NMDA immersion were statistically higher than those following low-dose NMDA immersion. High-dose (500 μmol/L) NMDA treatment resulted in a seizure score of 2-4 while low-dose (300 μmol/L) treatment resulted in a score of around 1 to 2 (Figure 4A). The same trend was identified for seizure onset time: The 500 μmol/L NMDA-treated group had a seizure onset time of fewer than 5 min, while the 300 μmol/L NMDA-treated group had a seizure onset time of nearly 10 min (Figure 4B). On the other hand, zebrafish immersed in high-dose NMDA after 10 μL 3 mg/kg MK-801, had a significantly reduced seizure score at all time points analyzed ($P < 0.001$) (Figure 4A) as well as a delayed seizure onset time (from approximately 5 min to 9 min) ($P < 0.001$) (Figure 4B). Resveratrol pretreatment also had a highly significant effect in lowering the seizure score from approximately 4 to 1 (Figure 4A) and delayed seizure onset time from around 5 min to about 10 min ($P = 0.0024$) (Figure 4B). These data indicate that seizures can be induced in zebrafish by immersion in NMDA in a slow and unremitting way and that MK-801 and resveratrol have anti-epileptic effects in this model system.

Comparison of the three administration modes

By comparing 1 h seizure scores and onset time of the three administration modes, we found high-dose NMDA immersion maintained the seizure score at about 2.5 to 3.5, the score caused by intraperitoneal injection was 4 to 5, and that following intravitreal injection was maintained at approximately 3.2 to 4.8 (Figure 5A). The trend in epilepsy caused by the three modes of administration generally increased initially and then decreased. In addition, the most severe stage of epilepsy caused by intraperitoneal injection of high-dose NMDA took about 30 min, while intravitreal injection took 40 min and immersion took 50 min. With regard to seizure onset time, immersion had the longest onset time of the three methods (approximately 6.3 min), which was followed by intraperitoneal injection (5.5 min). Intravitreal injection induced seizure-like behaviors within 3 min ($P < 0.001$) (Figure 5B). These data show that NMDA induces epilepsy-like behavior, while administration patterns alter seizure progression of

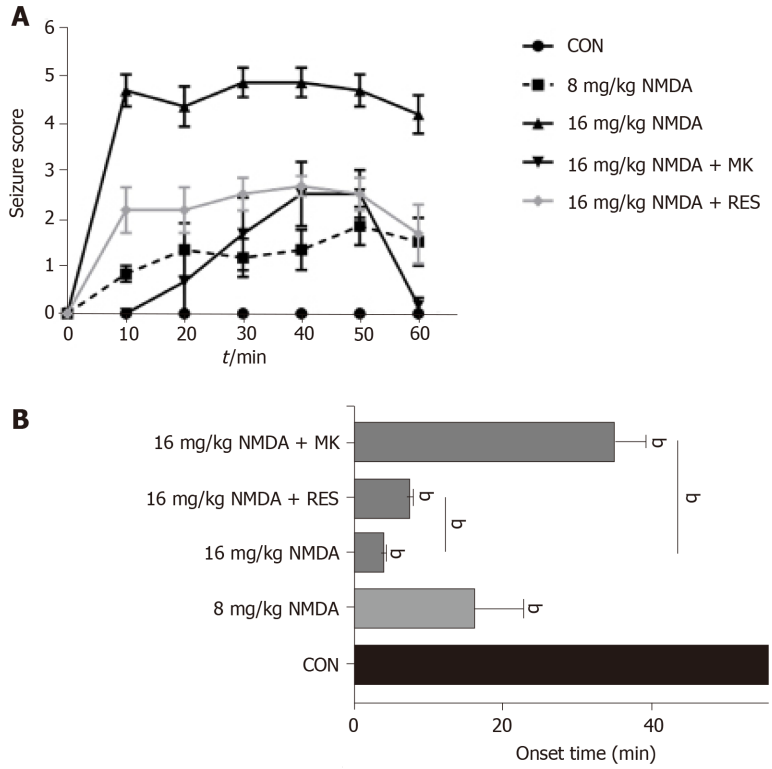

Figure 3 Intraperitoneal injection of N-Methyl-D-aspartic acid causes acute seizure-like behavior in zebrafish. Seizure activity scores and onset times following N-Methyl-D-aspartic acid (NMDA)-intraperitoneal injection (8 and 16 mg/kg, 50 mg/L resveratrol + 16 mg/kg NMDA, and 3 mg/kg MK-801 + 16 mg/kg NMDA). A: The mean seizure scores (± SE) for each group plotted against time after NMDA injection; B: The latency of seizure onset after NMDA injection. Bars represent mean ± SE of the time lag to the onset of the first convulsion in each group. Data were analyzed using one-way ANOVA and *t*-test for the different groups ($n = 6$) ($^{b}P < 0.01$ *vs* control). NMDA: N-Methyl-D-aspartic acid; RES: Resveratrol; MK: MK-801.

epilepsy diversely.

DISCUSSION

Numerous animal models have been used in epilepsy research. However, choosing the best experimental model mainly depends on the problem to be solved, the type of epilepsy simulated, whether it is consistent with the clinical characteristics, and whether it is simple and reliable. Therefore, selecting an appropriate and valuable animal model of epilepsy is undoubtedly an effective shortcut to better study the mechanism and treatment of epilepsy. Our preliminary experiment showed that intravitreal injection, intraperitoneal injection, and immersion all caused seizure-like behaviors in zebrafish. By using different NMDA concentrations, we utilized these three methods of drug delivery to establish seizure models associated with brain damage, which are also less harmful to adult zebrafish.

It has been reported that intravitreal injection of NMDA induces seizure-like behaviors in zebrafish. We found that intravitreal injection of NMDA was the most suitable for the establishment of an acute seizure model in zebrafish, as it caused seizure-like manifestations in a short time with a high concentration of NMDA in the central nervous system, which greatly reduces drug waste and shortens the time to disease. Intraperitoneal injection of NMDA did cause seizure-like behavioral changes in adult zebrafish, which was similar to analogous seizure models in rodents[37,38]. The effect caused by this method depends on the amount of NMDA injected due to strong intestinal absorptive capacity. When comparing the three modeling methods, immersion was less harmful to zebrafish in a short period of time and hence contributes to observing long-term and chronic epileptic behavior. As the drug was directly dissolved in water, and zebrafish can continuously take in the drug from the surrounding environment, the efficacy or toxicity of drugs is not observed in mammals and can be observed using this methd in a short time.

However, these three methods still have some limitations. Although intravitreal

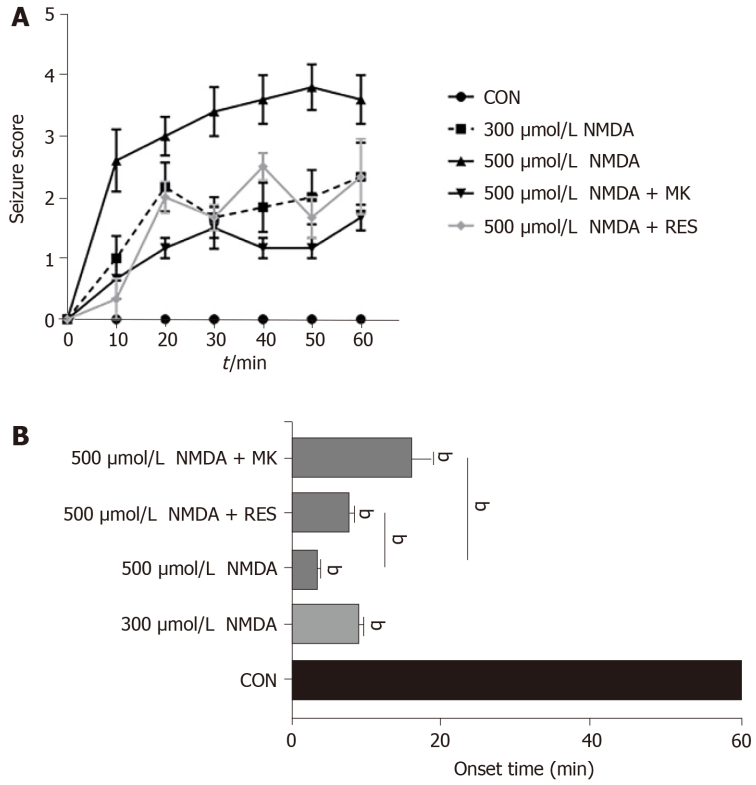

Figure 4 Immersion of N-Methyl-D-aspartic acid causes persistent seizure-like behavior in zebrafish. Seizure activity scores and onset times following N-Methyl-D-aspartic acid (NMDA)-immersion (300 μmol/L and 500 μmol/L NMDA, 40 mg/L resveratrol + 500 μmol/L NMDA, and 3 mg/kg MK-801 + 500 μmol/L NMDA). A: The mean seizure scores (± SE) for each group plotted against time after NMDA immersion; B: The latency of seizure onset after NMDA immersion. Bars represent mean ± SE of the time lag to the onset of the first convulsion in each group. Data were analyzed using one-way ANOVA and *t*-test for the different groups (n = 6) ($^aP < 0.05$, $^bP < 0.01$ *vs* control). NMDA: N-Methyl-D-aspartic acid; RES: Resveratrol; MK: MK-801.

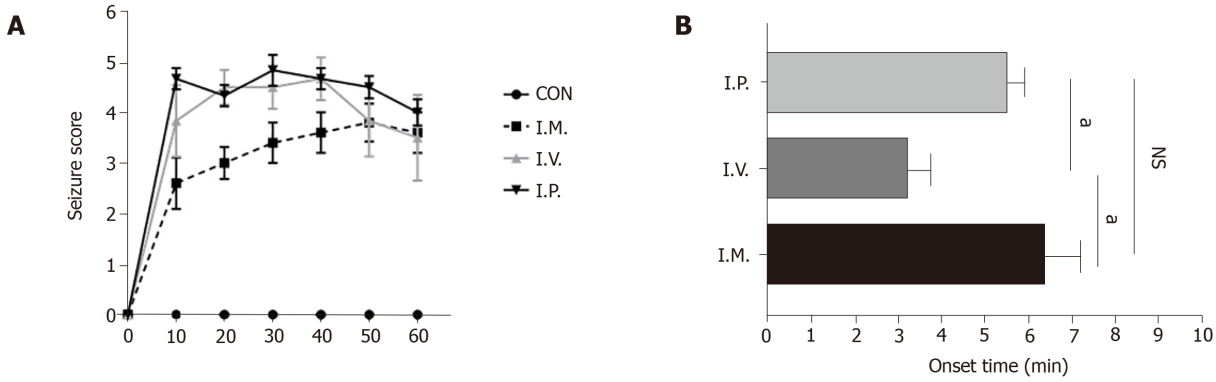

Figure 5 Comparison of the three administration routes on seizure score and onset time. Seizure activity scores and onset times following intravitreal injection, intraperitoneal injection and immersion with 0.5 mol/L, 16 mg/kg and 500 μmol/L N-Methyl-D-aspartic acid (NMDA), respectively. A: The mean seizure scores (± SE) for each route plotted against time after NMDA treatment; B: The latency of seizure onset after NMDA treatment. Bars represent mean ± SE of the time lag to the onset of the first convulsion using each method. Data were analyzed using one-way ANOVA and *t*-test for the different routes (n = 6) ($^aP < 0.05$). NMDA: N-Methyl-D-aspartic acid; I.V.: Intravitreal injection; I.P.: Intraperitoneal injection; I.M.: Immersion.

injection of NMDA exerts the same effect on zebrafish as other animal models, the difficulty of microinjection may hinder its application in drug screening. Therefore, to improve the potential of this model system, it is necessary to perfect each detailed procedure. In addition, the ocular pharmacokinetics of different drugs are sensitive to different clearance mechanisms[39], which is mainly affected by the permeability of retinal pigment epithelium. Understanding the clearance mechanism of different drugs in zebrafish will ensure the effectiveness of drug delivery, which provides a basis for ideal animal modeling and further drug screening. Intraperitoneal

administration is relatively easy, but it is worth noting that the procedure demands care in order to avoid injury to zebrafish organs such as the heart. With respect to the operational skills, immersion requires only control of the drug concentration.

According to the results of induced behavior changes, all three delivery modes were effective in delivering NMDA to the brain. Given that immersion lasts longer and takes effect later, the effects induced by intraperitoneal and intravitreal injection of NMDA on zebrafish appeared earlier. We hypothesized that intraperitoneal and vitreous injection would cause a sharp increase in NMDA concentration in the blood and target cells, resulting in acute pathophysiological changes, but NMDA concentration would then drop rapidly to baseline levels, and thus the dramatic response would quickly disappear. Of these three administration modes, the seizures induced by intravitreal injection appeared faster than the other two modes, which suggests that different administration routes into the zebrafish capillary network may have distinct effects of drug delivery. Intraperitoneal injection of NMDA has poor target specificity, rendering it hard to accumulate in the brain, and this drawback may cause uncontrollable damage to other non-targeted organs, such as the heart, which can interfere with the experiment results. In contrast, immersion can result in a relatively steady increase in NMDA concentration in the blood, which is enough to cause a long-term epileptic response; therefore, is more suitable for the model of sustained epilepsy. Unfortunately, due to the late onset time of seizures induced by drug immersion, it is not suitable for large-scale drug screening. At the operational level, immersion is the simplest and most convenient way to administer drugs, while intravitreal injection is relatively complicated but also the most effective way to establish the zebrafish model of brain disorder.

Antisense morpholine oligonucleotides (MOs) and hyperthermia have been used to construct zebrafish epilepsy models in previous studies. Although MOs can effectively interfere with protein synthesis of target genes, it can induce p53-dependent apoptosis and non-targeted cell-specific effects in gene expression, which in turn affect behavioral phenotype analysis[40]. The hyperthermia-induced zebrafish seizure model is more suitable for studying the mechanism of epileptic seizures *in vivo* and for acute seizure of chronic processes, but it does not show any persistence[41]. Both methods are appropriate for studying the mechanism of zebrafish seizures during innate or embryonic development. However, the methods we use can be applied to study the process of seizures in adulthood. Not only can they induce characteristic seizures which are similar to the reactions observed in mammalian seizures, but also emphasize the role of the zebrafish model in glutamate excitatory neurotransmission. For example, clomizole (a histamine receptor antagonist) is effective for gene-induced epilepsy of SCN1lab zebrafish (a model of Dravet syndrome caused by *SCN1a* mutation), a persistent drug-resistant epilepsy[42]. In addition, the methods we proposed can screen out the effect of psychotropic drugs and toxicity in the animal at a glance and reduce twists and turns in the drug development process.

It is noteworthy that one main defect in this NMDA-induced neurotoxicity model is that it only focuses on a single pathological mechanism (glutamate excitotoxicity) of epileptic seizures. Considering that the pathogenesis of human epilepsy is more complex, these models may not fully represent the pathogenesis of epilepsy; thus, may not be used to carry out clinical research on effective treatment methods for epilepsy[43]. In the current study, we only focused on seizure-like behavioral changes but did not carry out a specific analysis of pathological brain alterations or distortion of electrical signal transduction caused by excessive glutamate signaling. Therefore, further research is needed to fully establish the intravitreal administration route as a relevant model of epilepsy and other brain diseases. Although zebrafish seizure models are valuable for discovering anticonvulsants and studying ictogenesis, they are inadequate for the entire disease process. When studying epilepsy and screening anti-epileptic drugs, there is still a lack of epilepsy models that truly reflect the pathogenesis and characteristics of different forms of human epilepsy.

In conclusion, these results show that intravitreal injection of NMDA is an effective model for inducing acute epilepsy in zebrafish, and NMDA immersion can be used as a suitable model for persistent epilepsy. Additionally, intravitreal and intraperitoneal injection of NMDA may both be useful for modeling epilepsy. By comparing the three different drug administration patterns comprehensively, these models are valuable for identifying the potential mechanisms of epilepsy and drug screening. Last but not the least, our study provides convincing evidence for the potential application of MK-801 and resveratrol, a safe plant extract which is available for the treatment of epilepsy.

ARTICLE HIGHLIGHTS

Research background

Epilepsy is a complex neurological disorder characterized by recurrent, unprovoked seizures resulting from the sudden abnormal discharge of brain neurons. It leads to transient brain dysfunction, manifested by abnormal physical movements and consciousness. It can occur at any age, affecting approximately 65 million worldwide, one third of which are still estimated to suffer from refractory seizures. The molecular mechanism of epilepsy is still not fully understood; thus, there is a lack of effective clinical treatment. Therefore, building relevant preclinical models is imperative for screening therapeutics for this disease.

Research motivation

There is an urgent need for further establishment of seizure models in animals, including acute epilepsy models and persistent epilepsy models. These models could be used to study the mechanism of epilepsy and identify novel anti-epileptic therapeutics in the future.

Research objectives

The main objective was to compare three administration modes for establishing a seizure model caused by N-Methyl-D-aspartic acid (NMDA) in zebrafish.

Research methods

Three administration modes of NMDA, including immersion, intravitreal injection and intraperitoneal injection, were compared with regard to their effects on inducing seizure-like behaviors in adult zebrafish. We evaluated neurotoxicity by observing behavioral changes in zebrafish and graded those behaviors with a seizure score. Statistical analysis was performed based on records to calculate time points and duration of abnormal behavior in zebrafish. All data were analyzed by *t*-test using GraphPad PRISM 7.00. Analysis of variance was then performed to assess the differences in seizure and latency between experimental groups.

Research results

The three NMDA-administration methods triggered different patterns of the epileptic process in adult zebrafish. Seizure scores were increased after increasing NMDA concentration regardless of the mode of administration. However, the curve of immersion continuously rose to a high plateau (after 50 min), while the curves of intravitreal injection and intraperitoneal injection showed a spike in the early stage (10-20 min) followed by a steady decrease in seizure scores. Furthermore, pretreatment with resveratrol and MK-801 significantly delayed seizure onset time and lowered seizure scores.

Research conclusions

Intravitreal injection of NMDA was the most suitable route for establishing an acute epileptic model in zebrafish, while immersion with NMDA may be an appropriate method for inducing persistent seizures. Additionally, MK-801 and resveratrol showed strong anti-epileptic effects; thus, both of them may be clinically valuable treatments for epilepsy.

Research perspectives

Further study using our models to perform antiepileptic drug screening is necessary, and further work is needed to explore the mechanism of resveratrol against epilepsy.

REFERENCES

1 **Liao M**, Kundap U, Rosch RE, Burrows DRW, Meyer MP, Ouled Amar Bencheikh B, Cossette P, Samarut É. Targeted knockout of GABA-A receptor gamma 2 subunit provokes transient light-induced reflex seizures in zebrafish larvae. *Dis Model Mech* 2019; **12** [PMID: 31582559 DOI: 10.1242/dmm.040782]

2 **World Health Organization**. WHO's first Global Epilepsy Report. Available from: https://www.who.int/en/news-room/fact-sheets/detail/epilepsy

3 **Canzian J**, Müller TE, Franscescon F, Michelotti P, Fontana BD, Costa FV, Rosemberg DB. Modeling psychiatric comorbid symptoms of epileptic seizures in zebrafish. *J Psychiatr Res* 2019; **119**: 14-22 [PMID: 31542703 DOI: 10.1016/j.jpsychires.2019.09.007]

4 **Wang Z**, Luo Z, Li S, Luo Z, Wang Z. Anxiety screening tools in people with epilepsy: A systematic review of validated tools. *Epilepsy Behav* 2019; **99**: 106392 [PMID: 31521915 DOI: 10.1016/j.yebeh.2019.06.035]

5 **Jin M**, Zhang B, Sun Y, Zhang S, Li X, Sik A, Bai Y, Zheng X, Liu K. Involvement of peroxisome proliferator-activated receptor γ in anticonvulsant activity of α-asaronol against pentylenetetrazole-induced seizures in zebrafish. *Neuropharmacology* 2020; **162**: 107760 [PMID: 31493468 DOI: 10.1016/j.neuropharm.2019.107760]

6 **During MJ**, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* 1993; **341**: 1607-1610 [PMID: 8099987 DOI: 10.1016/0140-6736(93)90754-5]

7 **Rosenberg EC**, Patra PH, Whalley BJ. Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection. *Epilepsy Behav* 2017; **70**: 319-327 [PMID: 28190698 DOI: 10.1016/j.yebeh.2016.11.006]

8 **Alsharafi WA**, Xiao B, Li J. MicroRNA-139-5p negatively regulates NR2A-containing NMDA receptor in the rat pilocarpine model and patients with temporal lobe epilepsy. *Epilepsia* 2016; **57**: 1931-1940 [PMID: 27731509 DOI: 10.1111/epi.13568]

9 **Hui Yin Y**, Ahmad N, Makmor-Bakry M. Pathogenesis of epilepsy: challenges in animal models. *Iran J Basic Med Sci* 2013; **16**: 1119-1132 [PMID: 24494063]

10 **Chen HS**, Lipton SA. The chemical biology of clinically tolerated NMDA receptor antagonists. *J Neurochem* 2006; **97**: 1611-1626 [PMID: 16805772 DOI: 10.1111/j.1471-4159.2006.03991.x]

11 **Kemp JA**, McKernan RM. NMDA receptor pathways as drug targets. *Nat Neurosci* 2002; **5** Suppl: 1039-1042 [PMID: 12403981 DOI: 10.1038/nn936]

12 **Lipton SA**. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov* 2006; **5**: 160-170 [PMID: 16424917 DOI: 10.1038/nrd1958]

13 **Davies SJ**, Burhan AM, Kim D, Gerretsen P, Graff-Guerrero A, Woo VL, Kumar S, Colman S, Pollock BG, Mulsant BH, Rajji TK. Sequential drug treatment algorithm for agitation and aggression in Alzheimer's and mixed dementia. *J Psychopharmacol* 2018; **32**: 509-523 [PMID: 29338602 DOI: 10.1177/0269881117744996]

14 **Toscano CD**, Kingsley PJ, Marnett LJ, Bosetti F. NMDA-induced seizure intensity is enhanced in COX-2 deficient mice. *Neurotoxicology* 2008; **29**: 1114-1120 [PMID: 18834901 DOI: 10.1016/j.neuro.2008.08.008]

15 **Luo ZW**, Wang HT, Wang N, Sheng WW, Jin M, Lu Y, Bai YJ, Zou SQ, Pang YL, Xu H, Zhang X. Establishment of an adult zebrafish model of retinal neurodegeneration induced by NMDA. *Int J Ophthalmol* 2019; **12**: 1250-1261 [PMID: 31456914 DOI: 10.18240/ijo.2019.08.04]

16 **Soriano FX**, Papadia S, Hofmann F, Hardingham NR, Bading H, Hardingham GE. Preconditioning doses of NMDA promote neuroprotection by enhancing neuronal excitability. *J Neurosci* 2006; **26**: 4509-4518 [PMID: 16641230 DOI: 10.1523/JNEUROSCI.0455-06.2006]

17 **Vyklicky V**, Korinek M, Smejkalova T, Balik A, Krausova B, Kaniakova M, Lichnerova K, Cerny J, Krusek J, Dittert I, Horak M, Vyklicky L. Structure, function, and pharmacology of NMDA receptor channels. *Physiol Res* 2014; **63** Suppl 1: S191-S203 [PMID: 24564659]

18 **Bibliowicz J**, Tittle RK, Gross JM. Toward a better understanding of human eye disease insights from the zebrafish, Danio rerio. *Prog Mol Biol Transl Sci* 2011; **100**: 287-330 [PMID: 21377629 DOI: 10.1016/B978-0-12-384878-9.00007-8]

19 **Barbazuk WB**, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, McPherson JD, Johnson SL. The syntenic relationship of the zebrafish and human genomes. *Genome Res* 2000; **10**: 1351-1358 [PMID: 10984453 DOI: 10.1101/gr.144700]

20 **Chakraborty C**, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: a complete animal model for in vivo drug discovery and development. *Curr Drug Metab* 2009; **10**: 116-124 [PMID: 19275547 DOI: 10.2174/138920009787522197]

21 **Kari G**, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. *Clin Pharmacol Ther* 2007; **82**: 70-80 [PMID: 17495877 DOI: 10.1038/sj.clpt.6100223]

22 **Bouhenni RA**, Dunmire J, Sewell A, Edward DP. Animal models of glaucoma. *J Biomed Biotechnol* 2012; **2012**: 692609 [PMID: 22665989 DOI: 10.1155/2012/692609]

23 **Schirmer H**, Pereira TC, Rico EP, Rosemberg DB, Bonan CD, Bogo MR, Souto AA. Modulatory effect of resveratrol on SIRT1, SIRT3, SIRT4, PGC1α and NAMPT gene expression profiles in wild-type adult zebrafish liver. *Mol Biol Rep* 2012; **39**: 3281-3289 [PMID: 21706162 DOI: 10.1007/s11033-011-1096-4]

24 **Torres-Hernández BA**, Del Valle-Mojica LM, Ortíz JG. Valerenic acid and Valeriana officinalis extracts delay onset of Pentylenetetrazole (PTZ)-Induced seizures in adult Danio rerio (Zebrafish). *BMC Complement Altern Med* 2015; **15**: 228 [PMID: 26168917 DOI: 10.1186/s12906-015-0731-3]

25 **Alfaro JM**, Ripoll-Gómez J, Burgos JS. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. *Eur J Neurosci* 2011; **33**: 1252-1255 [PMID: 21375600 DOI: 10.1111/j.1460-9568.2011.07622.x]

26 **Menezes FP**, Rico EP, Da Silva RS. Tolerance to seizure induced by kainic acid is produced in a specific period of zebrafish development. *Prog Neuropsychopharmacol Biol Psychiatry* 2014; **55**: 109-112 [PMID: 24743104 DOI: 10.1016/j.pnpbp.2014.04.004]

27 **Fimbel SM**, Montgomery JE, Burket CT, Hyde DR. Regeneration of inner retinal neurons after intravitreal injection of ouabain in zebrafish. *J Neurosci* 2007; **27**: 1712-1724 [PMID: 17301179 DOI: 10.1523/JNEUROSCI.5317-06.2007]

28 **McGinn TE**, Mitchell DM, Meighan PC, Partington N, Leoni DC, Jenkins CE, Varnum MD, Stenkamp DL. Restoration of Dendritic Complexity, Functional Connectivity, and Diversity of Regenerated Retinal Bipolar Neurons in Adult Zebrafish. *J Neurosci* 2018; **38**: 120-136 [PMID: 29133431 DOI: 10.1523/JNEUROSCI.3444-16.2017]

29 **Szkudelski T**, Szkudelska K. Resveratrol and diabetes: from animal to human studies. *Biochim Biophys Acta* 2015; **1852**: 1145-1154 [PMID: 25445538 DOI: 10.1016/j.bbadis.2014.10.013]

30 **Yar AS**, Menevse S, Alp E, Helvacioglu F, Take G. The effects of resveratrol on cyclooxygenase-1 and cyclooxygenase-2 mRNA and protein levels in diabetic rat kidneys. *Mol Biol Rep* 2010; **37**: 2323-2331 [PMID: 19693696 DOI:

10.1007/s11033-009-9737-6]
31 **Marques FZ**, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. *Int J Biochem Cell Biol* 2009; **41**: 2125-2128 [PMID: 19527796 DOI: 10.1016/j.biocel.2009.06.003]
32 **Luo H**, Zhou M, Ji K, Zhuang J, Dang W, Fu S, Sun T, Zhang X. Expression of Sirtuins in the Retinal Neurons of Mice, Rats, and Humans. *Front Aging Neurosci* 2017; **9**: 366 [PMID: 29249955 DOI: 10.3389/fnagi.2017.00366]
33 **Ethemoglu MS**, Seker FB, Akkaya H, Kilic E, Aslan I, Erdogan CS, Yilmaz B. Anticonvulsant activity of resveratrol-loaded liposomes in vivo. *Neuroscience* 2017; **357**: 12-19 [PMID: 28577913 DOI: 10.1016/j.neuroscience.2017.05.026]
34 **Shetty AK**. Promise of resveratrol for easing status epilepticus and epilepsy. *Pharmacol Ther* 2011; **131**: 269-286 [PMID: 21554899 DOI: 10.1016/j.pharmthera.2011.04.008]
35 **Wang N**, Luo Z, Jin M, Sheng W, Wang HT, Long X, Wu Y, Hu P, Xu H, Zhang X. Exploration of age-related mitochondrial dysfunction and the anti-aging effects of resveratrol in zebrafish retina. *Aging (Albany NY)* 2019; **11**: 3117-3137 [PMID: 31105084 DOI: 10.18632/aging.101966]
36 **Reymond L**. Spatial visual acuity of the falcon, Falco berigora: a behavioural, optical and anatomical investigation. *Vision Res* 1987; **27**: 1859-1874 [PMID: 3445475 DOI: 10.1016/0042-6989(87)90114-3]
37 **Sierra S**, Alfaro JM, Sánchez S, Burgos JS. Administration of docosahexaenoic acid before birth and until aging decreases kainate-induced seizures in adult zebrafish. *Brain Res Bull* 2012; **88**: 467-470 [PMID: 22542883 DOI: 10.1016/j.brainresbull.2012.04.007]
38 **Yum MS**, Lee M, Woo DC, Kim DW, Ko TS, Velíšek L. β-Hydroxybutyrate attenuates NMDA-induced spasms in rats with evidence of neuronal stabilization on MR spectroscopy. *Epilepsy Res* 2015; **117**: 125-132 [PMID: 26452206 DOI: 10.1016/j.eplepsyres.2015.08.005]
39 **Zhang Y**, Bazzazi H, Lima E Silva R, Pandey NB, Green JJ, Campochiaro PA, Popel AS. Three-Dimensional Transport Model for Intravitreal and Suprachoroidal Drug Injection. *Invest Ophthalmol Vis Sci* 2018; **59**: 5266-5276 [PMID: 30383198 DOI: 10.1167/iovs.17-23632]
40 **Horie M**, Kotani T. Formation of mos RNA granules in the zebrafish oocyte that differ from cyclin B1 RNA granules in distribution, density and regulation. *Eur J Cell Biol* 2016; **95**: 563-573 [PMID: 27756483 DOI: 10.1016/j.ejcb.2016.10.001]
41 **Hunt RF**, Hortopan GA, Gillespie A, Baraban SC. A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. *Exp Neurol* 2012; **237**: 199-206 [PMID: 22735490 DOI: 10.1016/j.expneurol.2012.06.013]
42 **Baraban SC**, Dinday MT, Hortopan GA. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. *Nat Commun* 2013; **4**: 2410 [PMID: 24002024 DOI: 10.1038/ncomms3410]
43 **Staley K**. Molecular mechanisms of epilepsy. *Nat Neurosci* 2015; **18**: 367-372 [PMID: 25710839 DOI: 10.1038/nn.3947]

World Journal of Psychiatry

Submit a Manuscript: https://www.f6publishing.com

World J Psychiatr 2020 July 19; 10(7): 162-174

DOI: 10.5498/wjp.v10.i7.162

ISSN 2220-3206 (online)

SYSTEMATIC REVIEWS

Pimavanserin for the treatment of psychosis in Alzheimer's disease: A literature review

Shilpa Srinivasan, Rajesh R Tampi, Kripa Balaram, Arushi Kapoor

ORCID number: Shilpa Srinivasan 0000-0002-5852-2819; Rajesh R Tampi 0000-0002-0042-8344; Kripa Balaram 0000-0002-1943-7339; Arushi Kapoor 0000-0001-7020-1834.

Author contributions: All authors contributed to this work.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Manuscript source: Invited manuscript

Shilpa Srinivasan, Department of Neuropsychiatry and Behavioral Science, Palmetto Health USC Medical Group, University of South Carolina School of Medicine, Columbia, SC 29203, United States

Rajesh R Tampi, Department of Psychiatry and Behavioral Sciences, Cleveland Clinic Akron General, Akron, OH 44307, United States

Rajesh R Tampi, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH 44195, United States

Kripa Balaram, MD, Department of Psychiatry, MetroHealth, Cleveland, OH 44109, United States

Arushi Kapoor, Penn Memory Center at the Penn Neuroscience Center, Perelman Center for Advanced Medicine, Philadelphia, PA 19104, United States

Corresponding author: Shilpa Srinivasan, MD, Doctor, Professor, Department of Neuropsychiatry and Behavioral Science, Palmetto Health USC Medical Group, University of South Carolina School of Medicine, 3555 Harden Street Extension, Suite 301, Columbia, SC 29203, United States. shilpa.srinivasan@uscmed.sc.edu

Abstract

BACKGROUND

Alzheimer's disease (AD) is among the most prevalent forms of dementia in the world and neuropathological studies suggest similar high prevalence of mixed (AD + vascular) dementias. Approximately 25%-50% of individuals with AD develop psychosis sometime during their illness. The presence of psychosis in AD worsens outcomes. Currently there are no United States Food and Drug Administration (FDA) approved medications for the treatment of psychosis in AD. Pimavanserin, a novel atypical antipsychotic medication, was approved by the FDA for the treatment of hallucinations and delusions associated with Parkinson disease psychosis and is currently in clinical trials for the treatment of psychosis in AD.

AIM

To evaluate the existing literature regarding the use of pimavanserin for treating psychosis among individuals with AD.

METHODS

Received: February 13, 2020
Peer-review started: February 13, 2020
First decision: April 3, 2020
Revised: May 25, 2020
Accepted: June 10, 2020
Article in press: June 10, 2020
Published online: July 19, 2020

P-Reviewer: Hosak L, Seeman MV, Shiina A
S-Editor: Yan JP
L-Editor: A
E-Editor: Qi LL

A literature review of clinical studies of pimavanserin treatment for psychosis in individuals with AD was performed using the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines. Trials were identified by systematically searching PubMed, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and Scopus through October 2019. The 5-point Jadad scoring system was used to assess the methodologic quality of the randomized placebo-controlled trials.

RESULTS
A total of 499 citations were retrieved and pooled in EndNote and de-duplicated to 258 citations. This set was uploaded to Covidence for screening. Two separate screeners (Srinivasan S and Tampi RR) evaluated the titles, abstracts, and full text of eligible articles. Of the identified 258 abstracts, 98 articles underwent full text review and 2 publications from 1 randomized controlled trial (RCT) were included in the final analysis. The quality of evidence was assessed to be of good methodologic quality, scoring 4 out of 5 using the 5-point Jadad questionnaire with the Jadad Scoring calculation. This systematic review found only one RCT that evaluated the use of pimavanserin for the treatment of psychosis among individuals with AD. This phase 2 trial resulted in two publications, the second of which was a subgroup analysis from the original study. The evidence from these two publications showed that pimavanserin improves psychotic symptoms among individuals with AD when compared to placebo at week 6.

CONCLUSION
Pimavanserin may be a pharmacologic consideration for the treatment for psychosis in AD. Additional RCTs are needed to assess the evidence of effectiveness before pimavanserin is considered a standard treatment.

Key words: Pimavanserin; Alzheimer's disease; Psychosis; Psychotic disorders; Antipsychotic agents

Core tip: This systematic review was conducted to evaluate the evidence from randomized controlled clinical trials of pimavanserin for the treatment of psychosis in individuals with Alzheimer's disease (AD). Behavioral disturbances including psychosis are prevalent in AD, and have a significant impact on management and outcomes. There are currently no United States Food and Drug Administration approved medications for the management of behavioral disturbance in AD. Based on the findings of our systematic review, pimavanserin, a novel atypical antipsychotic, may be a new pharmacologic consideration for treating psychosis in individuals with AD.

Citation: Srinivasan S, Tampi RR, Balaram K, Kapoor A. Pimavanserin for the treatment of psychosis in Alzheimer's disease: A literature review. *World J Psychiatr* 2020; 10(7): 162-174
URL: https://www.wjgnet.com/2220-3206/full/v10/i7/162.htm
DOI: https://dx.doi.org/10.5498/wjp.v10.i7.162

INTRODUCTION

Alzheimer's disease (AD) is among the most prevalent form of dementia, affecting over two-thirds of the 50 million individuals living with dementia globally[1]. The combination of AD and vascular processes, also known as mixed dementia appears to also have a high prevalence according to neuropathological studies. Furthermore, overlap in clinical presentations can blur the distinction between AD and vascular dementia[2]. While cognitive impairment is a prominent clinical manifestation of AD, behavioral changes, also known as behavioral and psychological symptoms of dementia (BPSD) frequently occur. BPSD can include agitation, aggression, anxiety, depressed mood, apathy, and psychosis (delusions, hallucinations, paranoia)[3]. Between 25%-50% of individuals with AD develop psychosis, with variability in course, duration, and severity of psychotic symptoms[4,5]. The impact of psychosis is

far-reaching, affecting individuals and caregivers and is associated with higher rates of cognitive and functional decline, earlier time to institutionalization, higher treatment mortality, and caregiver burden respectively[6,7].

Available data indicates that psychosis in dementia occurs due to anatomical and biochemical changes within the brain[8]. Dysfunction of the adrenergic and serotonergic systems may also contribute to the behavioral symptoms of dementia[9]. Higher levels of norepinephrine in the substantia nigra and lower levels of serotonin in the presubiculum have been noted among individuals with psychosis in dementia when compared to non-psychotic individuals with dementia[10,11]. Neuropathologic changes that contribute to psychosis include the presence of neuritic plaques and tangles in the frontal and temporal cortices of these individuals[10,12,13]. Metabolic and perfusion imaging studies have demonstrated that psychosis in dementia correlates well with frontal, temporal and parietal lobe dysfunction[14-18]. A study of delusional misidentification symptoms (DMS) among individuals with AD found that individuals with DMS showed increased electroencephalograph delta-power over the right hemisphere and their computed tomography scans showed more severe right frontal lobe atrophy, and the number of their pyramidal cells in area CA1 was lower than in the patients without DMS[19]. There is also growing evidence that psychosis among individuals is higher among individuals who have relatives with AD and psychosis[20,21]. Psychosis is also more common among individuals with APOE3/4 genotype with more than threefold increase in the signs of depression and psychosis when compared with individuals with APOE 3/3 genotype or to control subjects[22]. Data also indicates that 5-HT2A receptor polymorphism 102-T/C and the 5-HT2C receptor polymorphism Cys23Ser are associated with the development of visual and auditory hallucinations among individuals with AD[23,24]. A study examining the association between selected polymorphisms in the dopamine receptor genes DRD1, DRD2, DRD3 and DRD4 and the presence of psychosis or aggressive behavior among individuals with AD found that psychosis and aggression were both significantly more frequent among the DRD1 B2/B2 homozygotes ($P < 0.02$), while psychosis was significantly more frequent in DRD3 1/1 or 2/2 homozygotes ($P < 0.05$)[25]. Another study found an association between the presence of psychotic symptoms and aggressive behavior and the DRD1 polymorphism and between the presence of psychosis, but not aggression and the DRD3 polymorphism[26]. Carriers of the DRD1 B2 allele were more likely to be aggressive or experience hallucinations whereas homozygous carriers of the DRD3 1 allele were more likely to experience delusions. These studies indicate that BPSD develops as a byproduct of the neurodegenerative disease process that manifests after a certain period, when the genetic factors assume greater significance in the brain[8].

Although non-pharmacological interventions are considered first line for BPSD, pharmacologic interventions may be warranted particularly when psychotic symptoms pose a potential threat to the individual or caregivers[27,28]. In the United States, there are currently no Federal Drug Administration (FDA)- approved medications for the treatment of psychosis in AD. While antipsychotic medications have been used to treat BPSD in individuals with AD, efficacy rates from randomized clinical trials have been shown to be modest[29,30]. In a systematic review conducted by Tampi *et al*[30] of 16 published meta-analyses evaluating antipsychotics in individuals with dementia, antipsychotics (convention and second generation/atypical) were found to demonstrate modest efficacy for the treatment of psychosis, agitation and aggression. However, significant limitations from an adverse effect profile were notable. The safety profile of antipsychotic medications must be considered, as they are associated with increased risk for death, cerebrovascular adverse events, sedation, falls, and pneumonia[29-32]. With these significant safety risk concerns, warnings regarding the use of antipsychotics in individuals with dementia-related psychosis have been issued by the United States FDA, the European Medicines Agency, and the United Kingdom Medicines and Healthcare Products Regulatory Agency[32]. Other pharmacologic agents, including cognitive enhancers (acetylcholinesterase inhibitors, memantine), glutamate modulators (dextromethorphan/quinidine), antidepressants and hormonal agents have been studied for agitation and aggression in AD with varying results and limited utility[7]. However, off-label use of antipsychotics, for dementia-related psychosis continues across different healthcare settings[33,34]. As a result, effective and safe pharmacological treatment of psychosis in AD remains an ongoing need.

Pimavanserin, an atypical antipsychotic medication, is a novel selective 5-HT2A inverse agonist with a low affinity for 5-HT2C receptors. Pimavanserin does not demonstrate clinically significant affinity to dopaminergic, histaminergic, muscarinic, or adrenergic receptors[35]. Subsequent to the results of a placebo-controlled six-week

clinical trial, pimavanserin received FDA approval in 2016 for the treatment of hallucinations and delusions associated with Parkinson disease psychosis (PDP)[36,37].

There is emerging evidence that pimavanserin is being trialed for the treatment of dementia-related psychosis[37]. Although published reviews have examined the benefits and risks of antipsychotics for the treatment of psychosis in dementia, to our knowledge, this is the first review that systematically evaluates the evidence from literature on the use of pimavanserin for psychosis in AD from randomized controlled trials (RCTs). In accordance with PICOS format (participants, interventions, comparisons, outcomes, and study design), all identified studies of RCTs in adult patients with AD who received pimavanserin for the treatment of psychosis in AD were included.

MATERIALS AND METHODS

A systematic literature search of clinical trials of pimavanserin was performed in accordance with the preferred reporting items for systematic reviews and meta-analyses guidelines[38]. Our study protocol was registered in the PROSPERO database of systematic reviews[39]. All identified studies of RCTs in adult patients with AD who received pimavanserin for the treatment of psychosis in AD were included. Studies of pimavanserin for the treatment of schizophrenia, dementia due to other etiologies, PDP, mood disorders, or other conditions were excluded. Literature involving nonhuman studies was also excluded.

We began our search with the Yale MeSH Analyzer (mesh.med.yale.edu), using key articles to refine the search strategy for the term pimavanserin. In each database we ran scoping queries followed by iterative refinement of the search strategy. Additional articles were identified by examining other systematic reviews, bibliographies, and pre-identified websites such as clinicaltrials.gov and publicly available internet searches (Google Scholar).

Literature searches were performed in the following databases from inception to October 25, 2019: PubMed, MEDLINE (Ovid), EMBASE (Ovid), Cochrane Central Register of Controlled Trials (Wiley), Web of Science Core Collection (Clarivate), and Scopus (Elsevier). No date or language restrictions were applied.

The databases were searched using both controlled vocabulary words and synonymous free text words for pimavanserin. The Cochrane highly sensitive search strategy was used to identify randomized trials in PubMed and Ovid databases[40]. The Cochrane RCT search strategy was adapted to identify trials in other electronic databases[41]. The search strategies were adjusted for the syntax appropriate for each database/platform. See supplementary material for MEDLINE search strategy (Supplementary Material).

RESULTS

The search retrieved a total of 499 references, which were pooled in EndNote and de-duplicated. This set was uploaded to Covidence for screening, which identified additional duplicates, leaving 258 for screening[42]. Two separate screeners (Srinivasan S and Tampi RR) evaluated the titles, abstracts, and full text of eligible articles. Of the identified 258 abstracts, 98 articles underwent full text review (Figure 1).

A total of two publications of pimavanserin among individuals with psychosis due to AD were identified and reviewed. Both were rated as being of good methodologic quality based on the five-point Jadad Score calculation[43]. This calculation assigns two points for randomization (one point for randomization and one point for description of system used to generate sequence of randomization), two points for blinding (description of, and method of blinding), and one point for description of withdrawals (Table 1). There was no funding for this review.

We identified and reviewed a total of two publications of pimavanserin for the treatment of psychosis in individuals with AD from a single trial. The trial used the aforementioned Jadad score calculation and was rated as being of good methodological quality (score of 4/5). The publications are described individually below and a brief summary of both publications is outlined in Table 2.

Study from Ballard et al, 2018

The 2018 study by Ballard *et al*[44] was a phase 2, randomized, double-blind, placebo-

Table 1 Jadad questionnaire

Jadad score calculation	
Item	Score (Yes = 1)
Was the study described as randomized	0/1
Was the method used to generate sequence of randomization described and appropriate?	0/1
Was the study described as double-blind?	0/1
Was the method of blinding described and appropriate?	0/1
Was there a description of withdrawals and dropouts?	0/1

Table 2 Summary of publications

Ref.	Year	Number of participants	Age (yr)	Setting	Comparators	Duration
Ballard *et al*[44]	2018	181 (pimavanserin n = 90; placebo n = 91)	≥ 50	Nursing homes	17 mg × 2 tablets pimavanserin *vs* placebo (2 tablets)	12 wk
Ballard *et al*[45]	2019	181 (pimavanserin n = 90; placebo n = 91)	≥ 50	Nursing homes	34 mg pimavanserin *vs* placebo	12 wk

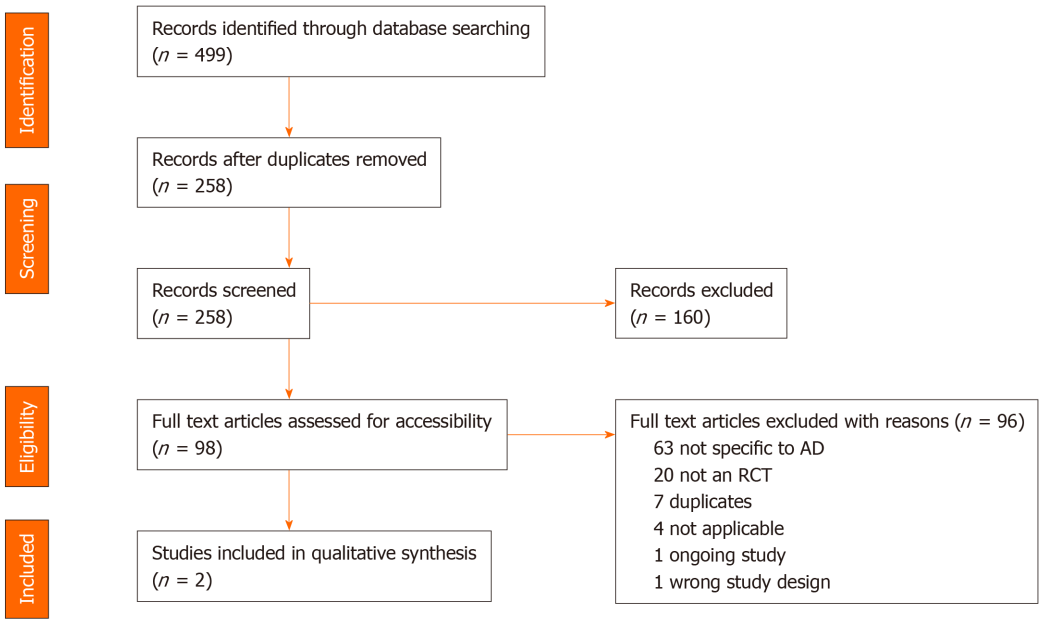

Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram. AD: Alzheimer's disease; RCT: Randomized controlled trial.

controlled single center trial. This study was conducted at nursing homes across the United Kingdom. Pimavanserin was compared to placebo in individuals with possible or probable AD and psychotic symptoms (visual or auditory hallucinations, delusions or both). This trial was 12 wk in duration and participants were aged 50 years or older. After completing screening and baseline evaluations, trial participants were randomly assigned in a 1:1 ratio to receive either pimavanserin or placebo. Pimavanserin dosing was two 17 mg tablets daily and placebo was also dosed as two tablets daily. Participants had to have resided in the nursing home for at least 4 wk in order to be eligible for the study. The degree of psychotic symptoms meeting eligibility criteria included clinical severity warranting antipsychotic treatment, and participant score of ≥ 4 for frequency × severity of hallucinations or delusions domains of the Neuropsychiatric Inventory-Nursing Home version (NPI-NH) psychosis scale, or a total combined score of ≥ 6 for hallucinations and delusions. Groups were stratified

based on baseline Mini-Mental State Examination (MMSE) total score of ≤ 6 or ≥ 6, and NPI-NH psychosis score of ≤ 12 or ≥ 12. Following screening, brief psychosocial therapy therapists evaluated participants during a 3-wk period and only individuals determined to require pharmacologic intervention progressed to study randomization, in an effort to minimize placebo response. This 3-wk period also served as a washout phase for participants who were taking antipsychotic medications.

After progressing through screening, individuals who m *et al*l study eligibility criteria at baseline (day 1) received a single dose of either pimavanserin (two 17 mg tablets) or placebo (two tablets). Participants continued to receive this regimen daily for 12 wk. Study visits were conducted at 2-wk intervals following baseline (day 1): days 15, 29, 43, 64, and 85, or at early termination. A telephone follow-up visit after the last dose of study medication was conducted for safety.

The primary outcome of this study was efficacy of pimavanserin *vs* placebo based on a change from baseline to week 6 in the NPI-NH psychosis score (hallucinations + delusions). Additional correlation analyses at week 6 included NPI-NH total score, NPI-NH agitation/aggression. AD Cooperative Study- Clinical Global Impression of Change (ADCS-CGIC), AD Cooperative Study- Activities of Daily Living (ADCS-ADL) total score, and the Cohen-Mansfield Agitation Inventory- Short Form (CMAI-SF) total score[45-48]. The assessment of behavioral symptoms at 6 wk and 12 wk constituted the secondary outcomes assessment. The ADCS-CGIC, NPI-NH agitation/aggression and sleep and nighttime domains, and the CMAI-SF total and subdomain scores were used as a measure of agitation. Additional subgroup analyses focused on stratified NPI- NH scores (< 12 or ≥ 12), baseline MMSE (< 6 or ≥ 6), sex, age (≤ 85 years or > 85 years). As well as for concomitant use of anti-dementia medication, selective serotonin reuptake inhibitor, and previous antipsychotic use. The MMSE was used to assess cognitive impairment, and the 1987 Unified Parkinson's Disease Rating Scale (UPDRS) Part III was used to measure extrapyramidal symptoms[49,50].

All study participants who received pimavanserin or placebo were included in the safety analysis, with safety outcomes measured over 12 wk. An adverse event checklist (all reported adverse events, those leading to study discontinuation, serious adverse events, and mortality), physical examinations, vital signs and electrocardiogram, and laboratory tests were also conducted.

Out of 345 screened participants, 181 were randomized to receive pimavanserin (n = 90) *vs* placebo (n = 91). Three participants in the pimavanserin group did not have a post-baseline NPI-NH score, and were excluded from the analysis. Of the 178 participants who were included, 160 completed 6 wk of treatment and 140 completed 12 wk. Of 26% of participants (n = 23) in the pimavanserin group and 20% of those (n = 18) in the placebo group withdrew over the 12-wk study period. The mean MMSE score was 10.3 in the pimavanserin group and 9.8 in the placebo group.

More women (80%-82%) than men and white individuals were represented among study participants. The mean age (years) in both groups was balanced (85.6 years in the pimavanserin group and 86.1 years in the placebo group). More study participants had an NPI-NH psychosis score < 12 (60%-61%). The mean MMSE score was 10.3 in the pimavanserin group and 9.8 in the placebo group.

The investigators noted that the improvement in the primary outcome (change in NPI-NH psychosis score) at week 6 was higher in the pimavanserin group (39.5% reduction) *vs* the placebo group (19.3% reduction). They did not observe any statistically significant differences between pimavanserin and placebo for the additional correlational analyses at week 6 or at week 12. Although not statistically significant (P = 0.063), a numerical difference of 5 points in the NPI-NH total score was observed for pimavanserin compared with placebo at week 6.

In regard to discontinuation due to adverse events, the investigators reported a 9% dropout rate (n = 8 of 90) in the pimavanserin group and 12% (n = 11 of 91) in the placebo group. Falls, urinary tract infection, and agitation were the most common adverse events across both treatment groups. While the frequency of falls (23%) and urinary tract infections (22% *vs* 28%) were similar across both treatment groups, more participants receiving pimavanserin experienced agitation compared to placebo. Agitation (21% *vs* 14%), aggression (10% *vs* 4%), and peripheral edema (8% *vs* 2%) were more prevalent in the pimavanserin treated group than with placebo. The investigators also did not note differences in vital signs, clinical laboratory results or physical examinations between groups. There were no discontinuations due to QTc prolongation. Furthermore, the investigators found no evidence of decline in cognition, function, global outcome, or motor symptoms over the 12-wk study period.

A summary of the findings from the 2018 Ballard *et al*[44] study, adverse events, and strengths/limitations are included in Table 3.

Table 3 Summary Ballard *et al*[44], 2018

Ref.	Outcomes	Tolerability	Limitations
Ballard *et al*[44], 2018	**Primary outcome**: **At week 6:** (1) Significant improvement in NPI-NH psychosis score (mean change was -3.76 points (SE 0.65) for pimavanserin group and -1.93 points (0.63) for placebo (mean difference -1.84 [95%CI: -3.64, -0.04]; P = 0.045) without negative effects on cognition or motor function; (2) Response (≥ 30% improvement) in 55% (pimavanserin) *vs* 37% (placebo); and (3) In NPI-NH < 12 subgroup: Mean change of the score from baseline to week 6 was -0.58 (95%CI: -2.10, 0.95) for pimavanserin *vs* -0.16 (-1.60 to 1.28) for placebo [mean difference -0.42 (95%CI: -2.52, 1.68)], Cohen's d = –0.77; P = 0.694. **At week 12:** No significant advantage for pimavanserin *vs* placebo was observed for the overall study population [treatment difference -0.51 (95%CI: -2.23, 1.21); P = 0.561]. **Secondary outcome: At weeks 6 and 12**: No significant differences between placebo and pimavanserin for ADCS–CGIC, NPI–NH agitation/aggression, NPI–NH sleep and nighttime behavior disorders, and CMAI–SF	Adverse events (pimavanserin *vs* placebo). **Most common:** (1) Agitation (21% *vs* 14%); (2) Aggression (10% *vs* 4%); (3) Falls (21% *vs* 21%); (4) Urinary tract infection (20% *vs* 25%); and (5) Peripheral edema (8% *vs* 2%). **Less common:** (1) Weight loss (-0.7 kg *vs* -0.1 kg); (2) QTc prolongation (9.4 ms *vs* -0.2 ms); and (3) Death (4 *vs* 4)	**Limitations:** (1) Study was not sufficiently powered to control for secondary outcomes; (2) Limited number of participants in severe psychosis subcategory or prior history of antipsychotic use; (3) Biomarker confirmation for patients diagnosed with Alzheimer's was not possible in the nursing home patients; (4) Possibility of trial participants inclusion of non-Alzheimer's type and mixed dementia; (5) High attrition rate: 26% (pimavanserin) and 20% (placebo); and (6) Absence of active comparator to assess efficacy/tolerability between pimavanserin and other antipsychotics. **Strengths**: (1) Rigorous diagnosis of psychosis – high completion rate at week 12; (2) Participants assessed in the community care homes providing access to the elderly population to be included in the clinical study; and (3) Researchers were able to study frail, elderly participants in their "natural" environment

CI: Confidence interval; NPI–NH: Neuropsychiatric Inventory-Nursing Home version; CMAI–SF: Cohen-Mansfield Agitation Inventory- Short Form; ADCS–CGIC: Alzheimer's disease Cooperative Study- Clinical Global Impression of Change.

Study from Ballard et al, 2019

This paper describes the outcomes of efficacy and tolerability of pimavanserin *vs* placebo in a subgroup of patients with severe psychosis associated with AD. Participants were part of the Phase 2 study summarized above and reported by Ballard *et al*[44] in 2018. The severity of psychosis was quantified by a cut-off score of ≥ 12 on the NPI-NH psychosis score. Participants in this subgroup analysis were nursing home residents with a baseline NPI-NH-NS score of ≥ 12, who were randomized to receive pimavanserin 34 mg or placebo daily over a 12-wk period. The primary endpoint at week 6 was the mean change from baseline on the NPI-NH-PS score. In addition, the investigators performed responder analyses, which was described as the observed proportions of individuals with an improvement from baseline at week 6. The investigators ascribed any missing values as non-responders.

The subgroup comprised of 57 participants (pimavanserin n = 27; placebo n = 30). Over 80% of participants in both pimavanserin and placebo groups were women. The average age range of participants was 85 years. A minority of participants in both groups had prior antipsychotic treatment (11.1% and 13.1%, pimavanserin *vs* placebo respectively). MMSE scores were similar across both pimavanserin and placebo subgroups (8.6 and 9.2 respectively) but were lower than in the overall study population described in the previous section.

In this subgroup, mean baseline NPI-NH psychosis scores were 15.3 (pimavanserin group) and 16.7 (placebo group). There was a statistically significant change in NPI-NH psychosis scores for pimavanserin *vs* placebo. Furthermore, the investigators reported that of 81% of study participants who had both hallucinations and delusions at baseline, pimavanserin was superior to placebo in treating these symptoms, with statistically significant improvement noted at week 6 for both domain scores (P = 0.046

for NPI-NH hallucinations, and P = 0.034 for NPI-NH delusions). While two-thirds of pimavanserin treated study participants had improvements in their NPI-NH psychosis score to < 6 (*vs* 32 % of placebo treated individuals) at week 6, 45.5% of both groups demonstrated this drop in NPI-NH score at week 12.

From a tolerability standpoint, there were no significant differences in the incidence of adverse events in the subgroup compared to the study population overall. Agitation, urinary tract infections and falls were the most common adverse events in the pimavanserin treated cohort. Over the 12-wk study period, the investigators noted that the change in MMSE score from baseline for the overall population across both treatment groups was minimal.

A summary of the findings from the 2019 Ballard *et al*[45] subgroup analysis, adverse events, and strengths/limitations are included in Table 4.

DISCUSSION

This review indicates that there is only one RCT that evaluated the use of pimavanserin for the treatment of psychosis among individuals with AD. This phase 2 trial resulted in two publications, the second of which was a subgroup analysis from the original study. The RCT was rated as having good methodological quality, scoring 4/5 on the Jadad score calculation. The evidence from these two publications indicates that pimavanserin improves psychotic symptoms among individuals with AD when compared to placebo at week 6. Additionally, among individuals with more severe psychotic symptoms (NPI-NH-NS score of ≥ 12), those individuals who were treated with pimavanserin had better outcomes than individuals receiving placebo. Pimavanserin was well tolerated in the study with the discontinuation rate due to adverse events being 9% in the pimavanserin group *vs* 12% in the placebo group. The investigators reported falls, urinary tract infection and agitation as the most common adverse events across both treatment groups with the frequency of falls (23%) and urinary tract infections (22% *vs* 28%) being similar across the treatment groups. Agitation (21% *vs* 14%), aggression (10% *vs* 4%) and peripheral edema (8% *vs* 2%) were more prevalent in the pimavanserin group when compared to placebo. The investigators also did not note any differences in vital signs, clinical laboratory results or physical examinations between the two groups. There were no discontinuations due to QTc prolongation. Additionally, treatment with pimavanserin did not result in decline in cognition, function, global outcome or motor symptoms over the 12-wk study period.

The strength of this review was the extensive review of multiple databases without any language restrictions. The limitation was that we restricted our review to only RCTs on the use of pimavanserin for the treatment of psychosis among individuals with AD. A review of clinicaltrials.gov found the SERENE study, conducted between 2016-2018 to evaluate the efficacy of pimavanserin 20 mg and 34 mg) *vs* placebo in the treatment of agitation and aggression in individuals with AD over 12 wk[51]. The primary outcome measure was change in the Cohen-Mansfield Agitation Inventory, while the secondary outcome measure was the Zarit Burden Interview, a measure of dementia caregiver stress[52]. While the original study design aimed to recruit 432 participants, 111 were randomized before recruitment was halted for business reasons. The investigators noted that the final study with 111 participants was no longer sufficiently powered to detect treatment effect.

Our review only found one RCT that met our inclusion criteria. The limitations of the study that we included in review were as follows: It was not sufficiently powered to control for secondary outcomes, there were limited number of participants in the severe psychosis subcategory or with a prior history of antipsychotic use, there were no biomarker confirmation for individuals with a diagnosis of AD who were living in the nursing homes and a high attrition rate among the pimavanserin (26%) and placebo (20%) groups. There strengths of the study were that there was a stringent criteria for the diagnosis of psychosis and there was participation from frail older adults living in community care homes in the study.

In a meta-analysis, Yasue *et al*[53] found that pimavanserin reduced the symptoms of hallucinations and delusions when compared to placebo [weighted mean differences (WMD) = -2.26, P = 0.005] among individuals with PDP. Additionally, pimavanserin was found to be superior to placebo in improving symptoms of hallucinations (WMD = -2.15, P = 0.001) and delusions (WMD = -1.32, P = 0.010) when considered independently. The authors did not find any significant difference between pimavanserin and placebo on the all-cause discontinuation rates for adverse events,

Table 4 Summary Ballard *et al*[45], 2019

Ref.	Outcomes	Tolerability	Limitations
Ballard *et al*[45], 2019	For overall population: Adjusted mean change from baseline at week 6 (adjusted mean, MMRM analysis) for the NPI-NH psychosis score was -3.76 (0.65) for pimavanserin *vs* -1.93 (0.63) for placebo (delta = -1.84, 95% confidence interval (CI) [-3.64, -0.04], Cohen's d = -0.32, P = 0.045); For patients with NPI-NH scores > 12: The mean change at week 6 was -10.15 (95%CI: -12.50, -7.80) for pimavanserin *vs* -5.72 (95%CI: -8.14, -3.30) for placebo (delta = -4.43 (95%CI: -7.81, -1.04), Cohen's d effect size of -0.73, P = 0.011); In the more severe subgroup, pimavanserin was superior to placebo at week 6 in treating both hallucinations (P = 0.046) and delusions (P = 0.034); At week 6, 66.7% of those in the pimavanserin group improved to an NPI-NH psychosis score < 6 *vs* 32.0% of those in the placebo group (difference = 34.7%); At week 12, 45.5% of both pimavanserin and placebo-treated patients had an NPI-NH psychosis score < 6; The proportion with a baseline NPI-NH psychosis score ≥ 12 achieving a response was significantly (P < 0.05) greater with pimavanserin *vs* placebo	Incidence of aggression was 14.3% in the severe psychosis subgroup *vs* 10.0% in overall population; Incidence of agitation was 17.9% in severe subgroup and 21.1% in general population; Other side effects included falls, UTI, contusion, respiratory tract infections, anemia, edema, cellulitis, anxiety, increase in urea or potassium	Small sample size in subgroup; Subgroup analysis was secondary
1	1	1	1

CI: Confidence interval; NPI–NH: Neuropsychiatric Inventory-Nursing Home version; UTI: Urinary tract infections.

death, Parkinson motor symptoms and the incidence of individual adverse events. Additionally, pimavanserin was found to be associated with less orthostatic hypotension when compared to placebo (risk ratio = 0.33, P = 0.008, number needed to harm = 17, P = 0.01). The investigators concluded that pimavanserin is beneficial for the treatment of symptoms of PDP and is well tolerated.

The data published by Institute for Safe Medication Practices indicated that there were a total 2236 adverse events reported from the use of pimavanserin in the 12 mo post-marketing observation period that ended March 2017, with hallucinations 487 (21.8%) drug ineffectiveness 333 (14.9%), confused state 258 (11.5%) and death 244 (10.9%) being the most commonly adverse events[54]. The United States FDA post-marketing review did not identify any new or unexpected safety findings with the use of pimavanserin[55]. In addition, the FDA did not find information that was inconsistent with the established safety profile for the drug concluding that the drug's benefits outweigh its risks among individuals with PDP reporting hallucinations and delusions.

In conclusion, the data is limited given only one published RCT to date has examined the use of pimavanserin for the treatment of psychosis among individuals with AD. While evidence from this study suggests pimavanserin is effective and tolerated, more rigorous trials are needed to establish evidence of effectiveness. However, given the identified risks of using antipsychotics among individuals with dementia, caution should be advised when using newer antipsychotic medications among individuals with dementia[56]. Additional larger studies of longer duration of treatment with positive outcomes will be needed prior to pimavanserin being adopted as a standard treatment option among individuals with AD who have psychosis.

ARTICLE HIGHLIGHTS

Research background

Alzheimer's disease (AD) is among the most prevalent forms of dementia in the world. Approximately 25%-50% of individuals with AD develop psychosis sometime during their illness. The presence of psychosis in AD worsens outcomes. Currently there are no United States Food and Drug Administration (FDA) approved medications for the treatment of psychosis in AD.

Research motivation

Pimavanserin, a novel atypical antipsychotic medication, was approved by the FDA for the treatment of hallucinations and delusions associated with Parkinson disease psychosis and is currently in clinical trials for the treatment of psychosis in AD.

Research objectives

This review evaluates the existing literature regarding the use of pimavanserin to treat psychosis among individuals with AD.

Research methods

A literature review of clinical studies of pimavanserin treatment for psychosis in individuals with AD was performed using the preferred reporting items for systematic review and meta-analysis guidelines. Trials were identified by systematically searching PubMed, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and Scopus through October 2019. The 5-point Jadad scoring system was used to assess the methodologic quality of the randomized placebo-controlled trials.

Research results

This systematic review found only one randomized controlled trial (RCT) that evaluated the use of pimavanserin for the treatment of psychosis among individuals with AD. This phase 2 trial resulted in two publications, the second of which was a subgroup analysis from the original study. The evidence from these two publications showed that pimavanserin improves psychotic symptoms among individuals with AD when compared to placebo at week 6.

Research conclusions

Limited evidence indicates that pimavanserin may be a pharmacologic consideration for the treatment for psychosis in AD. Additional RCTs are needed to assess the evidence of effectiveness before pimavanserin is considered a standard treatment.

Research perspectives

Additional RCTs are needed to assess the evidence of effectiveness before pimavanserin would be considered a standard treatment for psychosis in AD.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge Funaro MC (MS, MLS) for her assistance with the literature and database search for this scientific review.

REFERENCES

1 **Alzheimer's disease International**. World Alzheimer's Report 2018. [published September. 2018; cited 24 May 2020] Available from: https://www.alz.co.uk/research/world-report-2018

2 **Custodio N**, Montesinos R, Lira D, Herrera-Pérez E, Bardales Y, Valeriano-Lorenzo L. Mixed dementia: A review of the evidence. *Dement Neuropsychol* 2017; **11**: 364-370 [PMID: 29354216 DOI: 10.1590/1980-57642016dn11-040005]

3 **Steinberg M**, Shao H, Zandi P, Lyketsos CG, Welsh-Bohmer KA, Norton MC, Breitner JC, Steffens DC, Tschanz JT; Cache County Investigators. Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *Int J Geriatr Psychiatry* 2008; **23**: 170-177 [PMID: 17607801 DOI: 10.1002/gps.1858]

4 **Murray PS**, Kumar S, Demichele-Sweet MA, Sweet RA. Psychosis in Alzheimer's disease. *Biol Psychiatry* 2014; **75**: 542-552 [PMID: 24103379 DOI: 10.1016/j.biopsych.2013.08.020]

5 **Zhao QF**, Tan L, Wang HF, Jiang T, Tan MS, Tan L, Xu W, Li JQ, Wang J, Lai TJ, Yu JT. The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis. *J Affect Disord* 2016; **190**: 264-271 [PMID: 26540080 DOI: 10.1016/j.jad.2015.09.069]

6 **Zahodne LB**, Ornstein K, Cosentino S, Devanand DP, Stern Y. Longitudinal relationships between Alzheimer disease progression and psychosis, depressed mood, and agitation/aggression. *Am J Geriatr Psychiatry* 2015; **23**: 130-140 [PMID: 23871118 DOI: 10.1016/j.jagp.2013.03.014]

7 **Porsteinsson AP**, Antonsdottir IM. An update on the advancements in the treatment of agitation in Alzheimer's disease. *Expert Opin Pharmacother* 2017; **18**: 611-620 [PMID: 28300462 DOI: 10.1080/14656566.2017.1307340]

8 **McIlroy S**, Craig D. Neurobiology and genetics of behavioural syndromes of Alzheimer's disease. *Curr Alzheimer Res* 2004; **1**: 135-142 [PMID: 15975078 DOI: 10.2174/1567205043332180]

9 **Cummings JL**, Back C. The cholinergic hypothesis of neuropsychiatric symptoms in Alzheimer's disease. *Am J Geriatr Psychiatry* 1998; **6**: S64-S78 [PMID: 9581223 DOI: 10.1097/00019442-199821001-00009]

10 **Zubenko GS**, Moossy J, Martinez AJ, Rao G, Claassen D, Rosen J, Kopp U. Neuropathologic and neurochemical correlates of psychosis in primary dementia. *Arch Neurol* 1991; **48**: 619-624 [PMID: 1710105 DOI: 10.1001/archneur.1991.00530180075020]

11 **Victoroff J**, Zarow C, Mack WJ, Hsu E, Chui HC. Physical aggression is associated with preservation of substantia nigra pars compacta in Alzheimer disease. *Arch Neurol* 1996; **53**: 428-434 [PMID: 8624218 DOI: 10.1001/archneur.1996.00550050058024]

12 **Farber NB**, Rubin EH, Newcomer JW, Kinscherf DA, Miller JP, Morris JC, Olney JW, McKeel DW Jr. Increased neocortical neurofibrillary tangle density in subjects with Alzheimer disease and psychosis. *Arch Gen Psychiatry* 2000; **57**: 1165-1173 [PMID: 11115331 DOI: 10.1001/archpsyc.57.12.1165]

13 **Tekin S**, Mega MS, Masterman DM, Chow T, Garakian J, Vinters HV, Cummings JL. Orbitofrontal and anterior cingulate cortex neurofibrillary tangle burden is associated with agitation in Alzheimer disease. *Ann Neurol* 2001; **49**: 355-361 [PMID: 11261510 DOI: 10.1002/ana.72]

14 **Kotrla KJ**, Chacko RC, Harper RG, Doody R. Clinical variables associated with psychosis in Alzheimer's disease. *Am J Psychiatry* 1995; **152**: 1377-1379 [PMID: 7653698 DOI: 10.1176/ajp.152.9.1377]

15 **Starkstein SE**, Vázquez S, Petracca G, Sabe L, Migliorelli R, Tesón A, Leiguarda R. A SPECT study of delusions in Alzheimer's disease. *Neurology* 1994; **44**: 2055-2059 [PMID: 7969959 DOI: 10.1212/wnl.44.11.2055]

16 **Mentis MJ**, Weinstein EA, Horwitz B, McIntosh AR, Pietrini P, Alexander GE, Furey M, Murphy DG. Abnormal brain glucose metabolism in the delusional misidentification syndromes: a positron emission tomography study in Alzheimer disease. *Biol Psychiatry* 1995; **38**: 438-449 [PMID: 8672604 DOI: 10.1016/0006-3223(94)00326-x]

17 **Sultzer DL**, Mahler ME, Mandelkern MA, Cummings JL, Van Gorp WG, Hinkin CH, Berisford MA. The relationship between psychiatric symptoms and regional cortical metabolism in Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 1995; **7**: 476-484 [PMID: 8555751 DOI: 10.1176/jnp.7.4.476]

18 **Hirono N**, Mori E, Ishii K, Kitagaki H, Sasaki M, Ikejiri Y, Imamura T, Shimomura T, Ikeda M, Yamashita H. Alteration of regional cerebral glucose utilization with delusions in Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 1998; **10**: 433-439 [PMID: 9813789 DOI: 10.1176/jnp.10.4.433]

19 **Förstl H**, Besthorn C, Burns A, Geiger-Kabisch C, Levy R, Sattel A. Delusional misidentification in Alzheimer's disease: a summary of clinical and biological aspects. *Psychopathology* 1994; **27**: 194-199 [PMID: 7846237 DOI: 10.1159/000284869]

20 **Sweet RA**, Nimgaonkar VL, Devlin B, Lopez OL, DeKosky ST. Increased familial risk of the psychotic phenotype of Alzheimer disease. *Neurology* 2002; **58**: 907-911 [PMID: 11914406 DOI: 10.1212/wnl.58.6.907]

21 **Bacanu SA**, Devlin B, Chowdari KV, DeKosky ST, Nimgaonkar VL, Sweet RA. Heritability of psychosis in Alzheimer disease. *Am J Geriatr Psychiatry* 2005; **13**: 624-627 [PMID: 16009739 DOI: 10.1176/appi.ajgp.13.7.624]

22 **Ramachandran G**, Marder K, Tang M, Schofield PW, Chun MR, Devanand DP, Stern Y, Mayeux R. A preliminary study of apolipoprotein E genotype and psychiatric manifestations of Alzheimer's disease. *Neurology* 1996; **47**: 256-259 [PMID: 8710089 DOI: 10.1212/wnl.47.1.256]

23 **Sweet RA**, Nimgaonkar VL, Kamboh MI, Lopez OL, Zhang F, DeKosky ST. Dopamine receptor genetic variation, psychosis, and aggression in Alzheimer disease. *Arch Neurol* 1998; **55**: 1335-1340 [PMID: 9779662 DOI: 10.1001/archneur.55.10.1335]

24 **Holmes C**, Smith H, Ganderton R, Arranz M, Collier D, Powell J, Lovestone S. Psychosis and aggression in Alzheimer's disease: the effect of dopamine receptor gene variation. *J Neurol Neurosurg Psychiatry* 2001; **71**: 777-779 [PMID: 11723200 DOI: 10.1136/jnnp.71.6.777]

25 **Holmes C**, Arranz MJ, Powell JF, Collier DA, Lovestone S. 5-HT2A and 5-HT2C receptor polymorphisms and psychopathology in late onset Alzheimer's disease. *Hum Mol Genet* 1998; **7**: 1507-1509 [PMID: 9700207 DOI: 10.1093/hmg/7.9.1507]

26 **Sweet RA**, Pollock BG, Sukonick DL, Mulsant BH, Rosen J, Klunk WE, Kastango KB, DeKosky ST, Ferrell RE. The 5-HTTPR polymorphism confers liability to a combined phenotype of psychotic and aggressive behavior in Alzheimer disease. *Int Psychogeriatr* 2001; **13**: 401-409 [PMID: 12003247 DOI: 10.1017/s1041610201007827]

27 **Kales HC**, Lyketsos CG, Miller EM, Ballard C. Management of behavioral and psychological symptoms in people with Alzheimer's disease: an international Delphi consensus. *Int Psychogeriatr* 2019; **31**: 83-90 [PMID: 30068400 DOI: 10.1017/S1041610218000534]

28 **Gerlach LB**, Kales HC. Managing Behavioral and Psychological Symptoms of Dementia. *Psychiatr Clin North Am* 2018; **41**: 127-139 [PMID: 29412841 DOI: 10.1016/j.psc.2017.10.010]

29 **Ballard CG**, Gauthier S, Cummings JL, Brodaty H, Grossberg GT, Robert P, Lyketsos CG. Management of agitation and aggression associated with Alzheimer disease. *Nat Rev Neurol* 2009; **5**: 245-255 [PMID: 19488082 DOI: 10.1038/nrneurol.2009.39]

30 **Tampi RR**, Tampi DJ, Balachandran S, Srinivasan S. Antipsychotic use in dementia: a systematic review of

benefits and risks from meta-analyses. *Ther Adv Chronic Dis* 2016; **7**: 229-245 [PMID: 27583123 DOI: 10.1177/2040622316658463]

31 **Ballard C**, Howard R. Neuroleptic drugs in dementia: benefits and harm. *Nat Rev Neurosci* 2006; **7**: 492-500 [PMID: 16715057 DOI: 10.1038/nrn1926]

32 **Mittal V**, Kurup L, Williamson D, Muralee S, Tampi RR. Risk of cerebrovascular adverse events and death in elderly patients with dementia when treated with antipsychotic medications: a literature review of evidence. *Am J Alzheimers Dis Other Demen* 2011; **26**: 10-28 [PMID: 21282274 DOI: 10.1177/1533317510390351]

33 **Zuidema SU**, Johansson A, Selbaek G, Murray M, Burns A, Ballard C, Koopmans RT. A consensus guideline for antipsychotic drug use for dementia in care homes. Bridging the gap between scientific evidence and clinical practice. *Int Psychogeriatr* 2015; **27**: 1849-1859 [PMID: 26062126 DOI: 10.1017/S1041610215000745]

34 **Gellad WF**, Aspinall SL, Handler SM, Stone RA, Castle N, Semla TP, Good CB, Fine MJ, Dysken M, Hanlon JT. Use of antipsychotics among older residents in VA nursing homes. *Med Care* 2012; **50**: 954-960 [PMID: 23047785 DOI: 10.1097/MLR.0b013e31825fb21d]

35 **Acadia Pharmaceuticals, Inc**. Nuplazid (pimavanserin) prescribing information. San Diego, California, 2016. [cited 24 May 2020]. Available from: https://www.acadia-pharm.com/product/

36 **Cummings J**, Isaacson S, Mills R, Williams H, Chi-Burris K, Corbett A, Dhall R, Ballard C. Pimavanserin for patients with Parkinson's disease psychosis: a randomised, placebo-controlled phase 3 trial. *Lancet* 2014; **383**: 533-540 [PMID: 24183563 DOI: 10.1016/S0140-6736(13)62106-6]

37 **U.S. Food and Drug Administration**. Nuplazid (pimavanserin). 2016 [cited 28 October 2019]. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/207318orig1s000toc.cfm

38 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**: 264-269, W64 [PMID: 19622511 DOI: 10.7326/0003-4819-151-4-200908180-00135]

39 **Muchie KF**. PROSPERO International prospective register of systematic reviews. 2018

40 **Lefebvre C**, Manheimer E, Glanville J. In: Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Wiley Online Library; Searching for studies, 2011

41 **Cochrane Work**. Trusted evidence. Informed decisions.. Better health. 2011 [cited 28 October 2019] Available from: https://work.cochrane.org/pubmed

42 **Covidence**. Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. [cited 28 October 2019]. Available from: www.covidence.org

43 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12 [PMID: 8721797 DOI: 10.1016/0197-2456(95)00134-4]

44 **Ballard C**, Banister C, Khan Z, Cummings J, Demos G, Coate B, Youakim JM, Owen R, Stankovic S; ADP Investigators. Evaluation of the safety, tolerability, and efficacy of pimavanserin versus placebo in patients with Alzheimer's disease psychosis: a phase 2, randomised, placebo-controlled, double-blind study. *Lancet Neurol* 2018; **17**: 213-222 [PMID: 29452684 DOI: 10.1016/S1474-4422(18)30039-5]

45 **Ballard C**, Youakim JM, Coate B, Stankovic S. Pimavanserin in Alzheimer's Disease Psychosis: Efficacy in Patients with More Pronounced Psychotic Symptoms. *J Prev Alzheimers Dis* 2019; **6**: 27-33 [PMID: 30569083 DOI: 10.14283/jpad.2018.30]

46 **Schneider LS**, Olin JT, Doody RS, Clark CM, Morris JC, Reisberg B, Schmitt FA, Grundman M, Thomas RG, Ferris SH. Validity and reliability of the Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord* 1997; **11** Suppl 2: S22-S32 [PMID: 9236949 DOI: 10.1097/00002093-199700112-00004]

47 **Galasko D**, Bennett D, Sano M, Ernesto C, Thomas R, Grundman M, Ferris S. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord* 1997; **11** Suppl 2: S33-S39 [PMID: 9236950 DOI: 10.1097/00002093-199700112-00005]

48 **Cohen-Mansfield J**, Marx MS, Rosenthal AS. A description of agitation in a nursing home. *J Gerontol* 1989; **44**: M77-M84 [PMID: 2715584 DOI: 10.1093/geronj/44.3.m77]

49 **Folstein MF**, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189-198 [PMID: 1202204 DOI: 10.1016/0022-3956(75)90026-6]

50 **Fahn S, Elton RL, the Members of the UPDRS Development Committee**. Unified Parkinson's disease rating scale. Fahn S, Marsden CD, Calne DB, Goldstein M. *Recent developments in Parkinson's disease*. Florham Park, NJ: Macmillan Health Care Information 1987; 153-163

51 **ACADIA Pharmaceuticals Inc**. Study to Examine the Safety and Efficacy of Pimavanserin for the Treatment of Agitation and Aggression in Alzheimer's Disease (SERENE). [accessed 30 December 2019]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/study/NCT02992132?show_locs=Y ClinicalTrials.gov Identifier: NCT02992132/

52 **Zarit SH**, Reever KE, Bach-Peterson J. Relatives of the impaired elderly: correlates of feelings of burden. *Gerontologist* 1980; **20**: 649-655 [PMID: 7203086 DOI: 10.1093/geront/20.6.649]

53 **Yasue I**, Matsunaga S, Kishi T, Fujita K, Iwata N. Serotonin 2A Receptor Inverse Agonist as a Treatment for Parkinson's Disease Psychosis: A Systematic Review and Meta-analysis of Serotonin 2A Receptor Negative Modulators. *J Alzheimers Dis* 2016; **50**: 733-740 [PMID: 26757194 DOI: 10.3233/JAD-150818]

54 **Institute for Safe Medication Practices**. Hallucinations and Pimavanserin (NUPLAZID), a New Kind of Drug for Psychosis. 2017 [cited 24 December 2019]. Available from: URL: https://www.ismp.org/sites/default/files/attachments/2018-01/2017Q1_0.pdf

55 **U.S. Food and Drug Administration**. FDA Analysis Finds No New or Unexpected Safety Risks Associated with Nuplazid (Pimavanserin), a Medication to Treat the Hallucinations and Delusions of Parkinson's Disease Psychosis. 2018 September [cited 24 December 2019]. Available from: URL:

https://www.ismp.org/sites/default/files/attachments/2018-01/2017Q1_0.pdf
56 **Farlow MR**, Shamliyan TA. Benefits and harms of atypical antipsychotics for agitation in adults with dementia. *Eur Neuropsychopharmacol* 2017; **27**: 217-231 [PMID: 28111239 DOI: 10.1016/j.euroneuro.2017.01.002]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com