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## Approaches to Optimizing Dantrolene Neuroprotection for the Treatment of Alzheimer's Disease

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### Abstract

Alzheimer's Disease (AD), a neurodegenerative disorder with high incidence and mortality, is leading its way to the top of the list of the deadliest diseases without an effective disease-modifying drug. Ca<sup>2+</sup> dysregulation, specifically abnormal release of Ca<sup>2+</sup> via over activated ryanodine receptor (RyR), has been increasingly considered as an alternative upstream mechanism in AD pathology. Consequently, dantrolene, a RyR antagonist and FDA approved drug to treat malignant hyperthermia and chronic muscle spasms, has been shown to ameliorate memory loss in AD transgenic mice. However, the inefficiency of dantrolene to pass the Blood Brain Barrier (BBB) and penetrate the Central Nervous System needs to be resolved, considering its dose-dependent neuroprotection in AD and other neurodegenerative diseases. In this mini-review, we will discuss the current status of dantrolene neuroprotection in AD treatment and a strategy to maximize its beneficial effects, such as intranasal administration of dantrolene.

### Keywords

Alzheimer's disease; dantrolene; treatment; intranasal; calcium; blood brain barrier

## 1. INTRODUCTION

Neuroprotective drugs with the potential to repair neuronal damage or promote neurogenesis have been increasingly investigated for the treatment of AD [1]. *For example*, drugs such as GM-1 gangliosides and gacyclidine, which inhibit abnormal Ca<sup>2+</sup> influx, and

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### CONFLICT OF INTEREST

Dr. Huafeng Wei was once a member of the Advisory Board of Eagle Pharmaceutical Company for a one-day meeting in 2017 and the contract expired. Eagle Pharmaceutical Company produces and sells ryanodex, a new formula of dantrolene. Dr. Huafeng Wei is one of the inventors in a US provisional patent application entitled "Intranasal Administration of Dantrolene for Treatment of Alzheimer's Disease" filed on June 28, 2019 (Serial number 62/868,820) by the University of Pennsylvania Trustee. The patent application is also part of the research collaboration agreement between the University of Pennsylvania and Eagle Pharmaceutical Company who makes and sells ryanodex, a new formula of dantrolene.

### CONSENT FOR PUBLICATION

Not applicable.

methylprednisolone and carbamylated erythropoietin, which reduce neuroinflammation, have shown neuroprotective effects [2]. Nevertheless, almost all investigated drugs for AD treatment have yielded disappointing results in clinical trials [2, 3]. As  $\text{Ca}^{2+}$  dysregulation has been proposed as an alternative mechanism, researchers have been studying dantrolene as a potential therapeutic drug [4]. In this mini-review, we summarize the current status of dantrolene therapy in AD and strategies for optimizing its therapeutic effects while minimizing its long-term side effects.

## 2. PATHOLOGY OF ALZHEIMER'S DISEASE

In the 1990s, the amyloid cascade hypothesis demonstrated that the deposition of amyloid- $\beta$  ( $\text{A}\beta$ ) oligomers is the initial pathological event in AD, leading to the formation of amyloid plaques and degradation of the brain and memory [5]. Many research efforts aiming at eliminating amyloid plaques for correction of memory loss in AD have failed [3]. For instance, a recent phase III clinical trial in 2018, conducted by Biogen using a drug called Aducanumab, targeting  $\text{A}\beta$  oligomers, has been terminated because it failed to decrease amyloid load [6, 7]. Interestingly, the trial was reinitiated because reanalysis of the original data demonstrated beneficial effects of Aducanumab at a higher dose. This example is one of many failed clinical trials [8]. Currently, developing drugs for AD include some secretase inhibitors and anti- $\text{A}\beta$  vaccines which are also based on the amyloid cascade hypothesis that the production of amyloidogenic  $\text{A}\beta$  in the brain is neurotoxic and triggers neuron atrophy and ultimately leads to dementia [9]. The  $\gamma$ -secretase inhibitors such as Semagacestat and Avagacestat can reduce  $\text{A}\beta$  production in cultured neurons and in AD animals, while anti- $\text{A}\beta$  vaccines increase  $\text{A}\beta$  clearance [10–12]. However, none of these amyloid based therapeutic approaches yielded efficacy in clinical trials. More importantly, patients who received Semagacestat treatment showed an increased risk of skin cancer, and therefore Phase III trials of Semagacestat were discontinued [13]. These unsatisfactory therapeutic outcomes of Semagacestat and other secretase inhibitors may suggest that the time for therapeutic intervention was too late or  $\text{A}\beta$  may not be the origin of the disease. Thus, there are urgent and enormous needs for the development of new anti-AD therapeutic agents that target the proximal route of the AD. After decades of research investigating new potential AD drug treatments, we have learned a lot more about the pathogenesis and mechanisms of AD. The recent effective treatment of AD cognitive dysfunction by dantrolene in various animal models suggests that  $\text{Ca}^{2+}$  dysregulation in AD plays an important role in AD pathology and cognitive dysfunction [14]. Although the extensive studies focused on the amyloid cascade hypothesis failed in developing a new drug, it has taught us a lot more about AD mechanisms and suggestions of alternative ways for AD treatment.

Metabolic processing of mutated Amyloid- $\beta$  Precursor Protein (APP) is known to affect the function of RyR and lead to the formation of amyloid plaques, the defining marker of AD. Research work has shown that hydrolysis of APP generates oligomers and APP Intracellular Domains (AICD) [9], *via* the beta-secretase mediated amyloidogenic pathway [9]. Oligomer formation has been shown to decrease Endoplasmic Reticulum (ER)  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_{\text{er}}$ ) by creating channels on the ER membrane which allows  $[\text{Ca}^{2+}]_{\text{er}}$  to leak, thus, increasing the concentration of  $\text{Ca}^{2+}$  in cytosolic space ( $[\text{Ca}^{2+}]_{\text{c}}$ ) and the mitochondria ( $[\text{Ca}^{2+}]_{\text{m}}$ ) [9, 15, 16]. It should be noted that AD presenilin mutation and associated dysfunction has been

demonstrated to cause the loss of normal presenilin function and reduce  $\text{Ca}^{2+}$  release from ER, resulting in increased  $[\text{Ca}^{2+}]_{\text{er}}$  concentration in the ER [9]. AICD was found to alter and induce the expression of crucial signaling genes such as RyR, a  $\text{Ca}^{2+}$  regulator and release channel on the ER membrane [9, 15–17]. Amounting evidence and data indicate that the dysregulation or overexpression of  $\text{Ca}^{2+}$  signaling influences the onset of AD [9, 15, 16]. Therefore, a drug that can inhibit the pathological  $\text{Ca}^{2+}$  signaling is proposed to be neuroprotective in AD.

In recent years,  $\text{Ca}^{2+}$  dysregulation has been increasingly recognized as an important mechanism of AD pathology and cognitive dysfunction [9, 16, 18]. Studies have found that  $\text{Ca}^{2+}$  dysregulation increases Reactive Oxygen Species (ROS), impaired autophagy, neurodegeneration, synapse and cognitive dysfunction, and learning and memory deficits [19]. Evidence supporting  $\text{Ca}^{2+}$  dysregulation as the precursor to the neurodegenerative pathology and symptoms seen in AD has given the calcium hypothesis of AD widespread support [19]. The following drugs are currently being studied for their ability to correct  $\text{Ca}^{2+}$  dysregulation in AD [18]. Carvedilol, a RyR antagonist which stabilizes ER calcium signaling and is in phase IV clinical trial [20]; S18986, an AMPA receptor antagonist which regulates  $\text{Ca}^{2+}$  permeability and has been shown to be neuroprotective [21]. Nilvadipine [16], an L-type Voltage-Gated Calcium Channel (VGCC) antagonist that has shown to prevent  $\text{A}\beta$  accumulation and neurotoxicity when orally administered and is currently in phase III clinical trials [22]. The studies mentioned above show RyR as a target for treating AD. Dantrolene, a RyR antagonist and FDA approved drug to treat malignant hyperthermia and other chronic muscle spasms, *etc.* [23], has been investigated in several different AD animal models as a potential disease-modifying drug due to its ability to correct RyR [15, 24–26].

### 3. STUDIES USING DANTROLENE FOR AD TREATMENT

Considering the abnormally increased number of type-2 RyRs and function of RyRs in brains of AD patients or animals [25, 27], inhibition of dantrolene on the channel activation and opening [9] is expected to correct RyR-mediated calcium dysregulation and associated pathology of neurodegeneration, synapse structure damage and dysfunction as well as cognitive dysfunction [15, 25, 26]. Animal research studies that have used dantrolene for the treatment of AD have produced mixed results. In a study conducted by Zhang *et al*, double transgenic (APPPS1) and Wild Type (WT) mice were fed a 5mg/kg dantrolene solution twice a week from 2 to 8 months of age [26]. Their results indicated that long-term administration of dantrolene increased  $\text{A}\beta$  burden in APPPS1 *vs.* WT mice brains [26]. Unfortunately, this study did not investigate the effects of dantrolene on cognitive function. However, our laboratory and others have shown dantrolene to be neuroprotective in triple transgenic Alzheimer mice models (3xTg-AD) [24, 25, 28]. In one of our studies conducted by Peng *et al.* in 2012, mice of 2 to 5 months of age were treated initially with dantrolene through Intracerebroventricular (ICV) injection, then due to high mortality during the ICV administration, mice of 5 to 13 months of age received Subcutaneous (SQ) injections of dantrolene (5mg/kg) 3 times a week. After memory tests were conducted and the mice brains were analyzed, it was found that dantrolene improved memory in dantrolene treated 3xTg-AD mice in comparison to 3xTg-AD control mice. Additionally, dantrolene treated

3xTg-AD mice displayed decreased amyloid plaque levels in their brains when compared to control 3xTg-AD mice [25]. In a different study conducted by Oulès *et al.*, in 2012, APP (swe)-expressing (Tg2576) mice received a SQ injection of dantrolene and PBS solution for 3 months. Their results showed that dantrolene decreased A $\beta$  burden and ameliorated memory and learning deficits in dantrolene treated mice [24] (Table 1). Although there is not a significant amount of published papers that thoroughly investigate dantrolene therapy to treat AD, the current evidence shows its great potential. However, our studies along with several others have indicated that dantrolene is minimally efficient to penetrate into the CNS which is a limitation for trying to optimize its therapeutic effect in AD and other neurodegenerative diseases [24, 25, 29–31]. Given the research publications [24, 25, 28] discussed above, dantrolene has shown itself to be effective in treating AD; but the publications have failed to fully investigate the concentration and duration of dantrolene in the brain. In our recent study discussed below, we investigated whether intranasal dantrolene is more efficient when administered intranasally and is effective in treating AD as a disease-modifying drug [27, 32].

Studies using various administration routes demonstrated mixed results. Most studies found that dantrolene is capable of improving memory, even if amyloid plaques did not change. There is a lack of animal studies that investigated neuroprotective concentrations of dantrolene in the brain relative to effectiveness.

Another benefit of using dantrolene for treatment of AD is due to its minimal side effects, even when it is administered for prolonged periods [29]. Some common side effects that have been reported are headaches, dizziness, drowsiness, anorexia, diarrhea, light-headedness, nausea and vomiting. Dantrolene has been shown to cause liver damage, but it is only attributed to chronic oral use at a high dose [29]. Scientists have been working to maximize effectiveness of dantrolene neuroprotection and minimize its side effects.

#### 4. NEW APPROACH TO OPTIMIZE DANTROLENE NEUROPROTECTION

Intranasal administration of therapeutic drugs has been used to improve drug penetration into the CNS for agents that have limitations of passing the BBB [30, 33]. Due to dantrolene's short half-life [23, 34], an improved administration method needs to be used for reaching a constant and elevated brain/plasma ratio [23]. Based on research indicating that dantrolene is dose-dependent [23–25, 29–31], the intranasal approach would be beneficial to cross the BBB and penetrate the CNS at an optimal concentration and duration [35].

Our recent study indicated that, compared to oral administration of dantrolene, intranasal approach provided significantly higher peak concentration and longer duration in the brain [36].

In our recent study [32], WT and 5XFAD mice were randomly assigned into 5 groups: Control, intranasal (IN), Vehicle, IN Dantrolene, SQ vehicle, and SQ Dantrolene. Additionally, the groups were divided into subgroups of early treatment, beginning at 2 months of age, and late treatment, beginning at 6 months of age. Additionally, we carried out various tests after treatments to examine motor, memory and smell functions. Our results

suggest that in comparison to the oral approach, the intranasal administration of dantrolene significantly increased the concentration and duration in the brain [32] (Table 1). Intranasal dantrolene also achieved greater passage through the BBB and higher brain concentration than SQ administration [32]. Furthermore, intranasal dantrolene treated 5XFAD mice had an overall better effect than the SQ approach in ameliorating memory loss, even when treatment started after the full onset of AD pathology and cognitive dysfunction. Additionally, intranasal dantrolene after 9 months of treatment, produced tolerable side effects on liver structures and motor, smell and liver functions. Interestingly, it did not affect amyloid pathology significantly [32]. This new study suggests that intranasal administration of dantrolene may optimize the neuroprotective effects in the CNS and minimize its toxic effects in the peripheral system (Fig. 1). We are investigating and confirming the therapeutic effects and side effects of intranasal dantrolene in animal models more translational to AD patients before its clinical trials in patients.

Current studies on dantrolene show that it is neuroprotective but has limitations due to its poor permeability to access the CNS and BBB at effective concentrations. Although some studies indicated that dantrolene did not ameliorate A $\beta$  deposition and plaque formation significantly [26, 32], it was still consistent in improving memory and cognitive function overall [24]. Our first study in which we administered dantrolene intranasally has shown to be significantly more effective in treating AD in comparison to the SQ approach. The intranasal dantrolene approach is strongly advised to optimize the treatment of AD but requires more investigation before clinical use.

Another approach to ameliorate RyR over activation mediated neuropathology in AD is the development of new RyR modulators and inhibitors, that have similar effects to dantrolene, but can pass the BBB easily and produce minimal side effects. It has been shown that S-107 can ameliorate protein kinase A-mediated phosphorylation of the RyR and inhibit pathological RyR calcium leakage, providing neuroprotection and ameliorating cognitive dysfunction in a FAD animal model [37] Ideally, it would be beneficial if these new compounds are able to pass the BBB easily and inhibit RyRs in the brain and associated pathology, with minimal side effects after chronic treatment in AD patients [38].

## CONCLUSION

With disappointing failures on new effective drug development in Alzheimer's disease focusing on amyloid pathology, it is the time to change the strategies in this research area. With its promising neuroprotective effects targeting upstream calcium dysregulation in Alzheimer's disease, dantrolene, a clinically used muscle relaxant, has been repurposed to be a disease modifying drug for effective treatment of Alzheimer's disease. The novel approach of intranasal administration of dantrolene, instead of commonly used oral approach, further increase dantrolene brain concentrations and durations not only strengthen its therapeutic effects but minimize its side effects.

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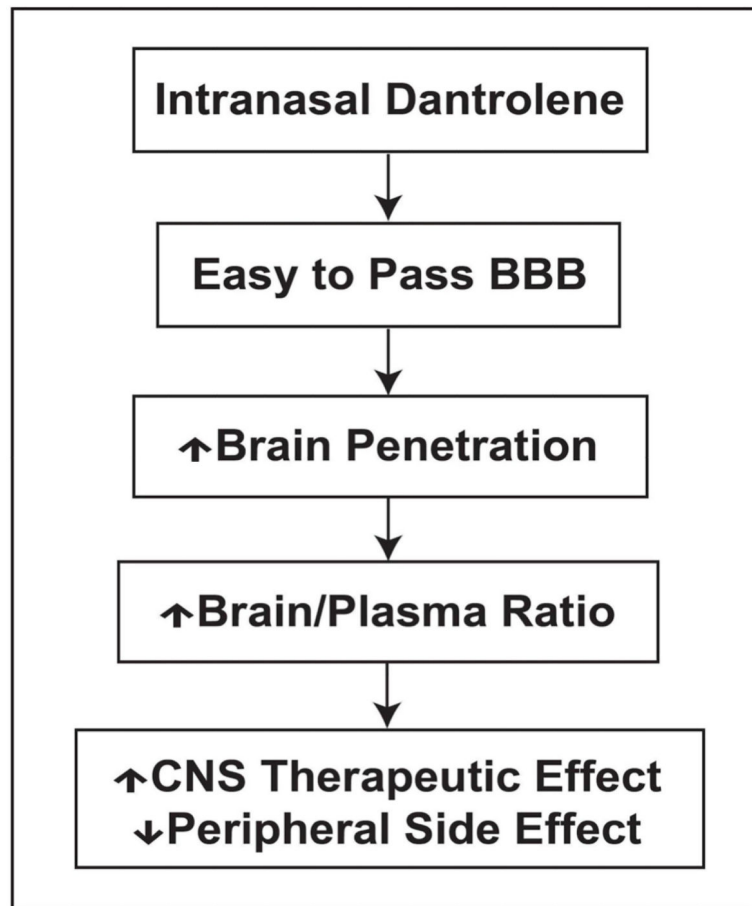
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**Fig. (1). Intranasal dantrolene maximizes CNS therapeutics but minimizes peripheral side effects.**

The increased dantrolene concentrations in CNS after intranasal administration yield effective therapeutic effects with a relatively lower dose and lower peripheral side effects.



Studies investigating therapeutic effects of dantrolene in treating AD through different administration routes.

**Table 1.**

Model Used	Administration Route	Dantrolene Brain Concentration	Outcome	References
3xTg-AD	ICV and SQ	ICV: 200–10,090 ng/g SQ: N/A	Decreased memory loss, Improved memory learning, and reduced A $\beta$ burden and plaque formation in the hippocampus.	[25]
Tg2576	SQ	N/A	Decreased A $\beta$ burden, senile plaque formation, and learning deficits.	[24]
APPS1	Oral	N/A	Increased amyloid load, loss of hippocampal excitatory synaptic markers, and neuronal atrophy.	[26]
5XFAD	IN	SQ at 20 min: 110–150 ng/g IN at 20 min: 110–250 ng/g SQ at 60 min: 90–110 ng/g IN at 60 min: 150–260 ng/g	Ameliorated memory loss, more effective than SQ, and did not change A $\beta$ burden in comparison to control.	[32]