

# The role of hydrogen in Alzheimer's disease

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## Abstract

Alzheimer's disease is one of the most common neurodegenerative diseases in the elderly. It is often manifested as learning and memory impairment, cognitive function decline, normal social and emotional disorders. However, for this high-risk common disease, there is currently no effective treatment, which has plagued many clinicians. As a new type of medical therapeutic gas, hydrogen has attracted much attention recently. As a recognized reducing gas, hydrogen has shown great anti-oxidative stress and anti-inflammatory effect in many cerebral disease models. It can ameliorate neuronal damage, maintain the number of neurons, prolong the lifespan of neurons, and ultimately inhibit disease progression. Therefore, the role and mechanism of hydrogen in the pathological process of Alzheimer's disease will be discussed in this paper.

**Key words:** hydrogen; Alzheimer's disease; experimental research; underlying mechanism; therapeutic implications; neuroprotection; anti-inflammation; anti-oxidative stress

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## INTRODUCTION

As is known to all, neurodegenerative diseases lead to the motor and cognitive disorder gradually. The characteristics of the neurodegenerative diseases is to reduce the number of cells in the brain and spinal cord, which are not renewable generally.<sup>1</sup> Alzheimer's disease (AD) is known to be the most common neurodegenerative disease. As its characteristics, the abnormal aggregation of  $\beta$ -amyloid (A $\beta$ ) and tau can be discovered.<sup>2</sup> Numerous studies on AD have been widely carried out in recent years. Moreover, researches of hydrogen therapy on neurodegenerative diseases have become a hotspot.

As the smallest gas molecule, molecular hydrogen is made of two protons and two electrons. In the past, it was considered to be physiologically inert in mammalian cells. In 1975, hydrogen was firstly discovered to show therapeutic effects in a skin squamous carcinoma mouse model.<sup>3</sup> In 2007, it was reported to show antioxidant properties by reducing hydroxyl radicals ( $\bullet$ OH), which aroused worldwide concern.<sup>4</sup> A large number of researches have been carried out to prove that hydrogen plays favorable effect in gastric diseases,<sup>5</sup> eye injury,<sup>6,7</sup> otology diseases,<sup>8</sup> oral diseases,<sup>9</sup> allergic rhinitis,<sup>10</sup> acute pancreatitis,<sup>11</sup> urinal system diseases,<sup>12</sup> skin disease,<sup>13</sup> hematopoietic system disorders,<sup>14,15</sup> mental diseases,<sup>16</sup> neurodegenerative diseases,<sup>17</sup> traumatic brain injury,<sup>18</sup> spinal cord ischemia/reperfusion injury,<sup>19</sup> and ischemic brain injury.<sup>20-22</sup> Hydrogen has been used in the study of so many disease models, so its safety also has received worldwide attention. The ignition point of hydrogen is 574°C, and it explodes with oxygen in the hydrogen concentration range of 4–75% (vol/vol).<sup>23</sup> Therefore, less than 4% of low concentration hydrogen which has demonstrated great efficacy is safe in researches and applications.<sup>24</sup> On the one hand, compared with other medical gases, hydrogen has a more prominent advantage in toxicity.

Even at high concentrations, hydrogen is still non-toxic<sup>25</sup> and has been used in diving applications.<sup>26</sup> Studies had showed that hydrogen inhalation did not have obvious adverse effects<sup>4</sup> and had no influence on blood pressure and other blood parameters such as pH, temperature.<sup>27</sup> This is consistent with the results of recent clinical trials in patients with cerebral infarction<sup>28</sup> and post-cardiac arrest syndrome.<sup>29</sup> Moreover, solubilized hydrogen such as hydrogen-rich water was recognized to be safe and easily administered in many researches.<sup>30-32</sup> A clinical trial conducted by Nagatani et al.<sup>33</sup> showed that intravenous solution of hydrogen-rich water was safe for patients who were suffering acute cerebral infarction, including those treated with tissue plasminogen activator. On the other hand, compared with other antioxidants, hydrogen showed less side effects since it only reduced  $\bullet$ OH.<sup>4</sup> In summary, the safety and non-toxicity of hydrogen have been confirmed in many studies. In our review, we focus on discussing the role of hydrogen in AD and summarizing possible molecular mechanisms.

## MECHANISMS OF THE HYDROGEN THERAPY IN ALZHEIMER'S DISEASE

AD is the most common neurodegenerative diseases which result in dementia.<sup>34,35</sup> AD patients often show decreased ability of learning and memory, impaired cognitive function, normal social and emotional disorders.<sup>36</sup> Inflammation and oxidative stress are recognized as the main causes of AD,<sup>37</sup> Parkinson's disease,<sup>38</sup> and other neurodegenerative diseases. Pathologically the deposition of A $\beta$  which results in neuritic plaques and the hyperphosphorylated tau protein are prominent features in AD.<sup>39</sup> The overproduction of A $\beta$  leads to dysfunction of mitochondrial complexes, which contribute to the overproduction of reactive oxygen species (ROS) and the depletion of adenosine triphosphate (ATP).<sup>40</sup> ATP is important



to neurotransmission and axonal transport, and it helps maintain the function of the ion channel and maintains the balance of ions inside and outside the cell.<sup>40</sup> So the depletion of ATP is the cause of mitochondrial dynamics damage.<sup>40</sup> Moreover, an increase in the amount of ROS causes a change in the pore size of the mitochondrial permeability transition pore, causing calcium ions to flow into the mitochondria, which in turn further exacerbates mitochondrial damage.<sup>40</sup> And ROS can also affect the function of the membrane to cause lipid peroxidation, promote cell apoptosis, and reduce the number of neurons.<sup>40</sup> Mitochondrial damage also caused by tau protein, which results in energy dysfunction, ROS production, and ultimately to affect synaptic function.<sup>41,42</sup> And ROS in turn accelerates tau hyperphosphorylation.<sup>40</sup> In summary, disorder of cholinergic function, amyloid cascade, oxidative stress, inflammation, excitotoxicity and steroid hormone deficiencies have been recognized to be the pathogenic mechanisms for AD.<sup>43</sup>

In addition, hydrogen molecules such as hydrogen-rich water, hydrogen-rich saline or hydrogen inhalation exhibit anti-inflammatory and anti-oxidant effects in many studies.<sup>44,45</sup> In many disease models, many pro-inflammatory cytokines are down-regulated such as nuclear factor- $\kappa$ B,<sup>46</sup> interleukin-1 $\beta$ , interleukin-6, interleukin-10, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), chemokine (C-C motif) ligand 2, interferon- $\gamma$ , and intercellular adhesion molecule-1, *etc.*<sup>47</sup> to exert anti-inflammatory effects by given hydrogen.<sup>1</sup> This effect also exists in the AD models.<sup>48</sup> In AD transgenic mouse model, the decline of nucleotide-binding domain leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) was proved to inhibit memory impairment and A $\beta$  deposition.<sup>49</sup> A current investigation reported that hydrogen could inhibit the activation of NLRP3 inflammasome in AD brains.<sup>50</sup>

Hydrogen can also stimulate energy metabolism to reduce neuronal damage. For example, it could up-regulate the expression of fibroblast growth factor 21<sup>51</sup> and ghrelin.<sup>52</sup> Lin et al.<sup>53</sup> found that hydrogen-rich water can stimulate AMPK-Sirt1-FoxO<sub>3</sub> pathway which could play a role in anti-oxidative stress, diminishing mitochondrial damage and acting as a neuroprotective agent, and neutralize ROS induced by A $\beta$ . Sirt1 could also induce autophagy, which plays a neuroprotective role in many neurodegenerative diseases.<sup>54</sup> So hydrogen can also protect cells by promoting autophagy in AD. And as is known to all, autophagy is an indispensable process to maintain cell homeostasis.<sup>55</sup> As members of the mitogen-activated protein kinase, phospho-p38 and c-Jun NH<sub>2</sub>-terminal kinase (JNK) participate in regulating cell survival.<sup>56,57</sup> Henderson et al.<sup>58</sup> reported that activated by oxidative stress P38K enhanced Bax phosphorylation and its translocation into mitochondria which led to apoptosis and neurodegeneration in AD brains. In many models, the results showed that that hydrogen water could suppress the activation of phospho-p38 and JNK.<sup>48,59,60</sup> This is consistent with other findings that molecular hydrogen can reduce neuronal apoptosis by inhibiting ROS-activated caspase signaling and protecting mitochondria.<sup>61</sup> In this regard, in AD, hydrogen treatment has anti-apoptotic effects.

Currently, Hou et al.<sup>50</sup> reported that hydrogen-rich water could improve cognition function in female transgenic AD mice by reducing the decline in brain estrogen levels, estrogen receptor (ER)  $\beta$ , and the expression of brain-derived neuro-

trophic factor (BDNF), but not in males without affecting  $\beta$ -amyloid precursor protein processing and A $\beta$  clearance. Furthermore, the suppression of inflammatory responses and oxidative stress was more pronounced in female AD mice than the males.<sup>50</sup> This suggests that hydrogen can also participate in the pathogenesis of AD by affecting the estrogen-ER  $\beta$ -BDNF signaling pathway. And estrogen can inhibit AD progression and neuronal damage by mitogen-activated protein kinase<sup>62</sup> and protein kinase C signaling pathway.<sup>63</sup> At the same time, BDNF and tyrosine kinase receptor B is thought to up-regulate the expression of genes which are associated with the differentiation of neuron, neuronal survival. Finally, it will improve synaptic plasticity and enhance learning and memory ability.<sup>64</sup> Moreover, the estrogen-ER $\beta$ -BDNF signaling pathway was associated with antioxidative and anti-inflammatory effects in AD.<sup>65</sup> 17 $\beta$ -oestradiol-induced signaling improved mitochondrial function which was related to ATP generation and oxidative phosphorylation.<sup>66</sup> The activation of ER $\beta$  signaling was also involved in ROS scavenging in pathological AD prevention.<sup>67</sup>

Therefore, anti-inflammatory, anti-oxidative stress, anti-apoptotic and the regulation of both autophagy and hormone signaling pathway are the main mechanisms of action of hydrogen.

## STUDIES OF HYDROGEN THERAPY IN ALZHEIMER'S DISEASE

Numerous studies have been carried out. Nagata et al.<sup>68</sup> reported that consumption of hydrogen water could prevent the decline of cognition, and maintain the proliferation of neural progenitors, and inhibit oxidative stress after chronic restraint stress in a mouse model of dementia. They observed the rise of malondialdehyde and 4-hydroxy-2-nonenal, which were recognized as oxidative stress markers enhanced by chronic restraint stress, was suppressed by using hydrogen water.<sup>68</sup> At the same time, hydrogen water restored the decrease in the number of proliferating cells in dentate gyrus after restraint stress.<sup>68</sup> Neurogenesis in the adult hippocampus keeps changing, which plays an important role in learning, memory and hippocampal plasticity.<sup>69,70</sup> The current study showed that cognitive impairments, pathological tau aggregation which were the characteristics of AD can be induced by the reduction of hippocampal neurogenesis.<sup>69</sup> This indirectly demonstrated that the effectiveness of hydrogen in the treatment of AD. In 2010, Li et al.<sup>71</sup> found that hydrogen-rich saline could reduce learning and memory impairments and neural inflammation which were induced by A $\beta$  in rats. They observed that hydrogen saline greatly improved learning memory and long-term potentiation (LTP), a form of synaptic plasticity related to learning and memory closely.<sup>71</sup> LTP was recognized to be blocked by A $\beta$  peptide oligomers rapidly and obviously.<sup>71</sup> Moreover, hydrogen-rich saline suppressed lipid peroxidation products, inflammatory factor like interleukin-6 and TNF- $\alpha$ , and the activation of astrocytes.<sup>71</sup> TNF- $\alpha$  also participated in the inhibition of LTP induced by A $\beta$ .<sup>71</sup> In the following year, the same team published that the protective effect of hydrogen-rich saline may be due to inhibition of the activation of JNK and NF- $\kappa$ B.<sup>48</sup> Another study showed that drinking hydrogen water for 30 days could exhibit the age-related impairment of



learning ability and memory in senescence-accelerated mouse prone 8 strain (SAMP8).<sup>72</sup> Additionally, senescence-accelerated prone mouse 8 showed the alleviation of the reduction of neurons in the hippocampus after being treated with hydrogen water for 18 weeks.<sup>72</sup> A similar study was discovered in 2015 by Kiyomi et al.<sup>73</sup> They used transgenic mice (DAL101) which lacked the activity of aldehyde dehydrogenase 2 as a dementia model. And they found that hydrogen-water could decrease oxidative stress and prevent the decline in cognition, learning and memory impairment and weaken neurodegeneration and the mean of lifespan of mice was extended. Moreover, they conducted a randomized clinical study which showed that hydrogen can greatly improve the cognition in the apolipoprotein E4 genotype carriers.<sup>73</sup> Recently, more and more evidence has shown that apolipoprotein E participates in anti-inflammatory, antioxidative and antiapoptotic effects during the process of brain injuries.<sup>74</sup> However, apolipoprotein E4 is thought to play a role in promoting oxidation, tau phosphorylation and the production of A $\beta$  in the pathological process of AD.<sup>40</sup> All experimental studies are shown in **Table 1**. Numerous studies are still underway, and clinical trials are being carried out gradually throughout the country.

## PROSPECTS

From an epidemiological point of view, women tend to have more extensive manifestations of dementia, and they experience more severe cognitive degeneration than men during AD progression.<sup>75</sup> And the loss of ovarian hormones is considered to be susceptible to AD in menopausal women.<sup>76</sup> Whereas hydrogen-rich water could reduce the decline in brain estrogen levels in females.<sup>50</sup> Therefore, hydrogen therapy for elderly women with AD may have a better effect. However, the specific mechanism about how hydrogen molecules inhibit the loss of estrogen remains unclear and further research is needed. On the one hand, Sirt1-FoxO3A axis might represent a new target for AD treatment. On the other hand in the pathology of AD, mitochondrial dysfunction is a non-negligible part, which may be another direction for the diagnosis and treatment of AD in the future. Currently, cathepsin B has been found that it links to NLRP3 inflammasome activation in AD.<sup>77</sup> So whether

cathepsin B is a target for hydrogen therapy also requires follow-up research.

## CONCLUSION

Hydrogen is a common non-toxic and safe medical gas, and its beneficial effects on AD have been confirmed in many studies, mainly through anti-inflammatory, anti-oxidative stress, anti-apoptotic and the regulation of both autophagy and hormone signaling pathway. Small-scale clinical studies have begun on the role of hydrogen in AD, so hydrogen is a new type of medical gas with great development prospects and we expect larger-scale clinical studies to be implemented.

### Author contributions

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**Table 1: Experimental studies of hydrogen in AD**

| Author                         | Animals/cells                     | Model   | Results  |
|--------------------------------|-----------------------------------|---|--|
| Hou et al. <sup>50</sup>       | Mice                              | AD  | Hydrogen-rich water inhibit NLRP3, and weaken the oestrogen-ER $\beta$ -BDNF signalling pathway.   |
| Lin et al. <sup>53</sup>       | Human neuroblastoma SK-N-MC cells | AD  | Hydrogen-rich water unregulated AMPK-Sirt1-FoxO3a pathway and neutralize excessive ROS to protect neuron.  |
| Nagata et al. <sup>68</sup>    | Mice                              | Dementia induced by chronic physical restraint stress | Molecular hydrogen inhibited the stress-induced learning and memory impairments.   |
| Li et al. <sup>71</sup>        | Rats                              | AD  | Hydrogen-rich saline improved memory function by inhibition of oxidative stress and reduction of interleukin-6 and TNF- $\alpha$ and the activation of astrocytes. |
| Gu et al. <sup>72</sup>        | Mice                              | AD  | Hydrogen water ameliorated neurodegeneration in hippocampus.   |
| Nishimaki et al. <sup>73</sup> | Mice                              | Dementia  | Molecular hydrogen improve the cognition in the apolipoprotein E4 geno-type carriers.  |

Note: AD: Alzheimer's disease; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; NLRP3: nucleotide-binding domain leucine-rich repeat and pyrin domain-containing protein 3; ROS: reactive oxygen species; ER: estrogen receptor; BDNF: brain-derived neurotrophic factor.





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