

# Assessing the Possible Influence of Residues of Ractopamine, a Livestock Feed Additive, in Meat on Alzheimer Disease

Frank S. Fan

Section of Hematology and Oncology, Department of Medicine, Ministry of Health and Welfare Taitung Hospital, Taitung, Taiwan

## Keywords

Alzheimer disease · Ractopamine · Amyloid- $\beta$  · Tau protein · Neurogenesis · Neuroinflammation

## Abstract

The feed additive ractopamine, a  $\beta$ -adrenergic agonist, has been approved for use in livestock for nearly 2 decades. Studies of its possible adverse effects in humans have concentrated exclusively on cardiovascular disease and cardiovascular functional disorders in the past. In this article, whether and how ractopamine may affect neurodegeneration, either to promote or to reduce the incidence of Alzheimer disease, will be discussed based on the recent controversial findings that  $\beta$ -adrenoreceptor activation not only can stimulate Alzheimer-pathogenic amyloid- $\beta$  accumulation but also are able to enhance hippocampal neurogenesis and ameliorate mouse memory deficits in independent laboratory studies. Furthermore, environmental enrichment has been found to prevent impairment of memory-related hippocampal long-term potentiation and microglia-mediated neuroinflammation induced by amyloid- $\beta$ . These beneficial effects are achieved mainly through enhanced  $\beta$ -adrenergic signaling and can be imitated by  $\beta$  agonist isoproterenol. Finally, it has been demonstrated that the  $\beta$ -adrenergic agonist salbutamol could bind directly to tau protein and interfere with the tau filament formation seen in

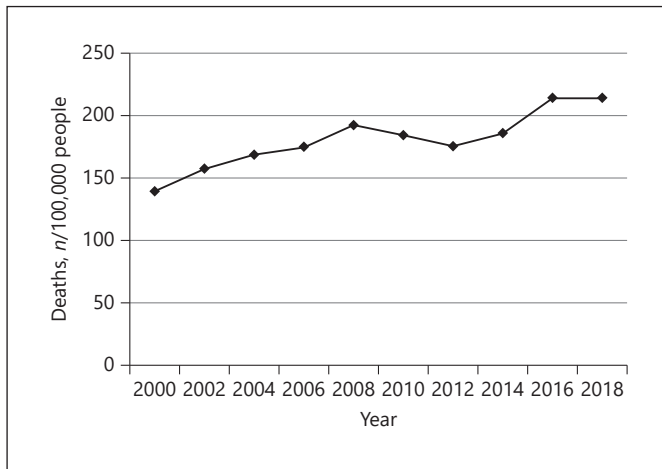
the prodromal phase of Alzheimer disease. These complex but interesting issues lead to contradictory speculations of possible effects of ractopamine residue in meat on Alzheimer disease. Hypotheses derived from this review surely deserve carefully designed laboratory investigations and clinical studies in the future.

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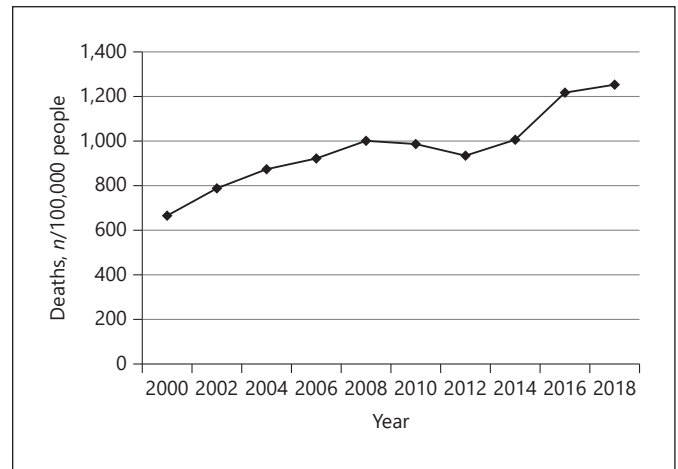
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## Introduction

Alzheimer disease, the main cause of dementia, is the most prevalent and rapidly growing neurodegenerative disorder around the world, with incidence rates rising exponentially with age and increasing notably after 65 years [1]. The diagnosis of probable and possible Alzheimer disease chiefly depends on clinical symptoms and modern image studies such as magnetic resonance imaging and positron emission tomography, while a definite diagnosis usually needs autopsy-confirmed characteristic pathologic findings of amyloid neuritic plaques and neurofibrillary tangles [2, 3]. There is currently no curative or disease-modifying treatment for Alzheimer disease, although a number of acetylcholinesterase inhibitors and *N*-methyl-D-aspartate receptor antagonists could provide symptomatic improvement of cognition



**Fig. 1.** Annual Alzheimer death rates (per 100,000 people) by year in the age group 75–84 years in the USA. Data are from the Alzheimer’s Association [16].



**Fig. 2.** Annual Alzheimer death rates (per 100,000 people) by year in the age group 85 years or older in the USA. Data are from the Alzheimer’s Association [16].

impairment [4]. Nonetheless, these now approved therapies are not able to change the rate of cognitive decline in a long-run aspect [5]. Preventative strategies thus seem to be an urgently important issue. Regarding risk factors for Alzheimer disease, in addition to genetic, vascular, lifestyle, physical, and metabolic ones, such as hypertension, diabetes, hypercholesterolemia, and smoking [6], is there any other agent worthy of concern? Herein, ractopamine, a  $\beta$ -adrenoreceptor agonist widely used as a feed additive in beef and pork production in the USA but prohibited in European Union and China [7], is the focus of interest.

### Mechanism of Alzheimer Disease and Novel Therapies

Though the exact causes of Alzheimer disease are not certain and very complex, hypothesized mechanisms have been developed indicating that the gradually accumulated extracellular neuritic plaques composed of amyloid- $\beta$  peptide ( $A\beta$ ) interact with the intracellular neurofibrillary tangles formed by the tau protein and elicit neuroinflammation which finally leads to neurodegeneration [8].  $A\beta$  aggregation derives from alterations of different  $A\beta$  species production from amyloidogenic amyloid- $\beta$  protein precursor ( $A\beta$ PP) and their clearance, whereas the main pathogenic modification of tau is post-translational hyperphosphorylation. Based on the recent understanding of the pathogenic roles that  $A\beta$  and tau

play in the very beginning stage of Alzheimer disease’s natural course, therapeutic modalities aimed at inhibition of  $A\beta$  and tau formation have been eagerly designed and tested [9]. Nevertheless, these candidate drugs will require successful clinical trials to prove their benefits and this will take time [10]. The biggest concern is the timing of treatment. Even if novel agents could inhibit  $A\beta$  and tau formation at once, the effects on dementia brains already suffering from long-term neurodegeneration would be doubtful.

### $\gamma$ -Secretase and $\beta$ -Adrenoreceptor

The amyloidogenic  $A\beta$  is generated from the transmembrane  $A\beta$ PP through successive cleavage by  $\beta$ - and  $\gamma$ -secretases while  $A\beta$ PP processed by  $\alpha$ -secretase results in nonamyloidogenic products. Dyshomeostasis of  $A\beta$  leads to synaptotoxic soluble oligomers capable of phosphorylating tau and insoluble fibrils which aggregate into plaques [11]. The  $\gamma$ -secretase is a complex composed of 4 subunits, i.e., presenilin, nicastrin, anterior pharynx-defective 1, and presenilin enhancer 2 [12]. Mutations of  $A\beta$ PP and presenilin genes are found to be closely associated with familial Alzheimer disease, highlighting the key role of  $\gamma$ -secretase in the pathogenesis of Alzheimer disease [13]. Further disclosing of the underlying causes of Alzheimer disease, research on regulation of  $\gamma$ -secretase by environmental factors has revealed that activation of  $\beta$ -adrenoreceptor can enhance  $\gamma$ -secretase activity in cell

culture, and treatment with  $\beta$ -adrenoreceptor agonist increased cerebral amyloid plaques in a mouse model, suggesting that abnormal  $\beta$ -adrenoreceptor activation by stress might contribute to A $\beta$  accumulation in Alzheimer disease [14].

### **Hypothesis: Ractopamine and Amyloid- $\beta$**

The global population is still growing, especially in Asia, creating a need for food production. It is therefore not strange to see livestock producers in the USA, Canada, Brazil, and Mexico using the  $\beta$ -adrenoreceptor agonist ractopamine to increase the growth rate and percentage of leanness of their finishing cattle and swine [15]. Although ractopamine has been approved for use under the no-observed-adverse-effect level for nearly 20 years, as a  $\beta$ -adrenoreceptor activator its chronic potential influence on A $\beta$  accumulation and thus the incidence of Alzheimer disease deserve careful investigation. Figures 1 and 2 were created based on annual Alzheimer's death rates by age and year in the USA from 2000 to 2018 [16]. As we can see, in both age groups (i.e., 75–84 years and 85 years or older) there seems to be a progressive elevation trend across the time period. This trend is coincidentally compatible with the hypothesis that consumption of meat containing ractopamine might stimulate A $\beta$  production and aggregation in the brain, silently resulting in Alzheimer disease.

### **Enhancement of Neurogenesis**

Nonetheless, the role of  $\beta$ -adrenoreceptor in the pathogenesis of Alzheimer disease is still quite controversial. Contrary to what has been mentioned above, activation of the  $\beta_2$ -adrenoreceptor by the  $\beta_2$ -adrenergic agonist clenbuterol in transgenic mice carrying chimeric mouse/human A $\beta$ PP and mutant human presenilin 1, an Alzheimer disease animal model, significantly stimulated hippocampal neurogenesis and reversed memory deficits [17]. Another selective  $\beta_2$ -adrenergic agonist, i.e., salmeterol, when chronically administered in mice, exerts a prosurvival effect of neuroblasts and beneficially modulates hippocampal neuroplasticity [18]. Accordingly, ractopamine residue in meat probably would improve symptoms of dementia in Alzheimer disease rather than worsen them. However, the net results of a speculated increased A $\beta$  accumulation and presumed enhanced neurogenesis clearly require more intelligent laboratory work and long-

term clinical observation in order for a definite conclusion to be reached.

### **Environmental Enrichment against Amyloid- $\beta$**

Furthermore, impairment of memory-related hippocampal long-term potentiation by A $\beta$  was found to be protected by an enriched environment for rodents through activation of the  $\beta_2$ -adrenergic receptor and the cAMP/PKA signaling pathway [19, 20]. Environmental enrichment by adding novel objects and running wheels to mouse cages constitutes a behavior paradigm that models cognitive activity of humans. Researchers of these studies disclosed that the  $\beta_{1/2}$  agonist isoproterenol prevented the inhibition of A $\beta$  on long-term potentiation while nonselective  $\beta$  antagonist decreased the beneficial effects of environmental enrichment. In addition to long-term potentiation, microglia-mediated neuroinflammation, a key factor in the progression of Alzheimer disease, could also be blocked by environmental enrichment through enhanced  $\beta$ -adrenergic signaling [21, 22]. All of these results lead to speculation that ractopamine, a  $\beta_{1/2}$  agonist, might play an ameliorating role in the development of Alzheimer disease.

### **Inhibition of Tau Aggregation**

Besides A $\beta$ , neurofibrillary tangle from tau filaments is considered to be another therapeutic target in Alzheimer's disease due to its pathogenic roles. A variety of modalities have been developed to inhibit tau expression, disturb tau aggregation, stabilize microtubules, and remove tau with immunotherapy, but their satisfactory efficacy and safety have yet to be proven clinically [23]. Interestingly, the  $\beta$ -adrenergic agonist salbutamol was recently found to be capable of inhibiting tau aggregation in an experiment adopting high-throughput synchrotron radiation circular dichroism and conventional circular dichroism spectroscopy. Surprisingly, the mechanism of tau inhibition by salbutamol is not through  $\beta$ -adrenoreceptor activation but rather an interfering effect by direct binding of salbutamol to prefibrillar tau [24]. It is thus hard to deduce whether ractopamine also has a molecular docking action similar to that of salbutamol because different molecules have different conformations and hydrogen bonding orientations.

## Discussion and Conclusion

In the past, the safety concern of ractopamine as a feed additive has mainly focused on its risk of carcinogenesis, genotoxicity, and cardiovascular disorders, with almost no attention being paid to chronic neurologic influence [25]. It is hoped that this review will help to arouse interest in this aspect, especially the occurrence of Alzheimer disease. Although there is much controversy surrounding the real impact of ractopamine on neurodegeneration, thanks to the progress of nuclear medicine imaging techniques, early amyloid accumulation and neuroinflammation induced by A $\beta$  or tau can now be detected by position emission tomography, facilitating the diagnosis of Alzheimer disease in its prodromal phase [26, 27]. In countries where people consume meat containing ractopamine, close follow-up and monitoring with such modern imaging devices is highly recommended in order to inves-

tigate and survey the possible influence of ractopamine on the central nervous system to see whether it is a friend or foe.

## Conflict of Interest Statement

The author has no conflict of interests to report.

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## Author Contributions

F.S.F. alone conceptualized the idea, collected the literature, and wrote this review.

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