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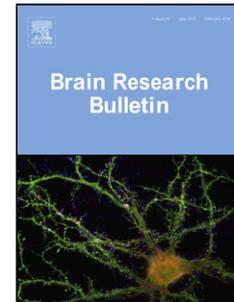
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A systematic literature review of the effect of insulin sensitizers on the cognitive symptoms of Alzheimer's Disease in transgenic mice.

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Highlights

- Impaired insulin signalling is seen in the brain of those with Alzheimer's Disease.
- A literature review was performed to assess the effect of insulin sensitizers.
- Improvements in cognition, Amyloid β and tau pathology were demonstrated in mice.
- Studies of a greater quality, accuracy and internal validity are needed in future.

Abstract

Clinical implication

Alzheimer's Disease (AD) presents as a gradual decline in cognitive function, with its characteristic pathology consisting of Amyloid β ($A\beta$) accumulation and hyperphosphorylated tau. Impaired insulin signalling was recently found in the brain in AD, and shown to increase AD pathology. Similar insulin resistance is found in type 2 diabetes and is currently treated with insulin sensitizers (IS).

Aims and Method

The aim of this literature review was to evaluate whether IS could effectively reduce AD's characteristic pathology and symptoms in models of AD in transgenic mice. The efficacy of an IS (Metformin, Rosiglitazone or Pioglitazone) at improving each characteristic in transgenic mice was evaluated.

Results

A variety of cognitive tests and measures of pathology were utilized to assess these outcomes, hindering comparison. Improvements in cognition, learning and $A\beta$ pathology were demonstrated by some papers, and all papers reported a decrease in tau phosphorylation, but no effect on total tau levels.

Key Words: Insulin sensitizer, Metformin, Thiazolidinediones, Alzheimer's Disease

Abbreviations

$A\beta$ - Amyloid β

AD - Alzheimer's Disease

IS - Insulin Sensitizers

MET - Metformin

PTZ - Pioglitazone

RTZ - Rosiglitazone

T2D - Type 2 Diabetes Mellitus

TZD – Thiazolidinediones

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Introduction

Alzheimer's Disease (AD) is a debilitating neurodegenerative disorder that contributes to 60-70% of dementia cases, is rapidly rising in prevalence, yet still has no preventative treatment (1). The characteristic pathology of A β aggregation and hyperphosphorylation of tau, found upon AD's discovery in 1907, are still its most identifiable features (2). However, they have yet to be linked to one precipitating factor, with the aetiology of this disease still not fully understood. Recently, research has been considering impaired insulin signalling as the trigger, due to its association with accelerated cognitive decline and neurodegeneration in AD (3–5).

This link between insulin deficiency and resistance and the development of AD pathology has led to the proposition that AD may be a neuro-endocrine disorder, and thus could be termed 'Type 3 Diabetes' (T3D) (6). Whilst there is considerable overlap between the physiological processes of Type 2 Diabetes (T2D) and AD, the impaired insulin signaling seen in AD is unique and brain-specific, so cannot be classed as either Type 1 Diabetes (T1D) or T2D. Potentially the strongest evidence in support of T3D is the effect on the cognitive and pathological features of AD following the use of insulin sensitizers (IS).

Currently, treatment for AD effects cognitive and non-cognitive symptoms, but are not disease modifying (7). A promising new avenue for therapeutic intervention, is to combat the impaired insulin signalling found in the brain in AD (8). Peripheral insulin resistance in Type 2 Diabetes Mellitus (T2D) is prevented by the use of Insulin Sensitizers (IS). The main drugs currently used as IS are Metformin (MET) and the Thiazolidinediones (TZD); Rosiglitazone (RTZ) and Pioglitazone (PTZ).

Aims

Consequently, the aim of this study was to evaluate whether, if used in AD, IS may be effective at counteracting insulin resistance in the brain and thus repairing insulin signalling (9). This would potentially reduce the cognitive decline and characteristic AD pathology shown to be a consequence of this pathological process. The effect of IS on A β aggregation, hyperphosphorylation of tau and cognitive decline were of primary interest in this review. If

IS are shown to be effective at reducing the pathology and symptoms of AD in mice, it is possible that with further trials and investigations they could be effective in treating AD clinically.

Methods

Data sources

In order to identify relevant papers, a comprehensive literature search of Ovid Medline, Ovid Embase, Pubmed, Scopus and Cochrane library was performed on February 19th 2017. For each database, a combination of search criteria was used: Alzheimer's Disease AND Metformin, in addition to Alzheimer's Disease AND Thiazolidinediones OR Pioglitazone OR Rosiglitazone. Relevant papers were then identified by ascertaining their relevance and conformity to a set inclusion and exclusion criteria.

Inclusion Criteria:

- Models of AD in Transgenic mice
- Insulin sensitizers
 - -Metformin
 - -Rosiglitazone
 - -Pioglitazone
- Effect on cognitive function, A β or tau pathology assessed
- Papers between 2007 and 2017
- Animal intervention studies

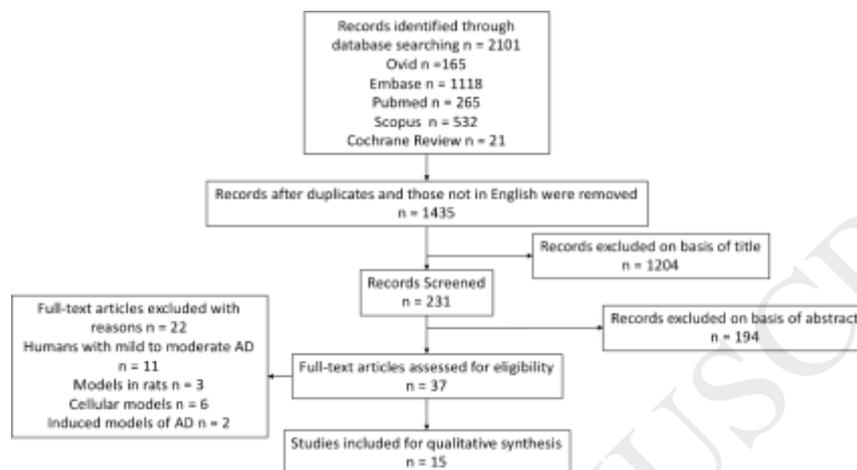
Exclusion Criteria:

- Human patients with mild to moderate AD or type 2 diabetes
- Other anti-diabetic drugs or insulin sensitizers used in combination with another treatment
- Effect on mild cognitive impairment or mood dysfunction
- Systematic reviews

Paper selection

Overall, the databases produced 2101 potentially relevant papers. Only 15 papers ultimately met the inclusion criteria for this review (Figure 1).

Figure 1. PRISMA flow diagram



Flow diagram depicting the results of the systematic literature search and paper selection process undergone.

The 15 papers included in this paper were:

Metformin Papers:

Barini et al. Metformin promotes tau aggregation and exacerbates abnormal behavior in a mouse model of tauopathy. *Mol. Neurodegener.* 2016; **11**, 16.

DiTacchio et al. Metformin treatment alters memory function in a mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* 2015; **44**, 43–48.

Son et al. Metformin Facilitates Amyloid-beta Generation by beta- and gamma-Secretases via Autophagy Activation. *J. Alzheimers. Dis.* 2016; **51**, 1197–1208.

Matthes et al. The anti-diabetic drug metformin improves cognitive impairment and reduces amyloid-beta in a mouse model of Alzheimer's disease. *Alzheimer's Dement.* 2015; **11**, P845.

Rosiglitazone Papers:

Yu et al. Insulin sensitizers improve learning and attenuate tau hyperphosphorylation and

neuroinflammation in 3xTg-AD mice. *J. Neural Transm.* 2015; **122**, 593–606.

O'Reilly and Lynch. Rosiglitazone improves spatial memory and decreases insoluble A β 1–42 in APP/PS1 mice. *J. Neuroimmune Pharmacol.* 2009; **7**, 140–144.

Escribano et al. Rosiglitazone reverses memory decline and hippocampal glucocorticoid receptor down-regulation in an Alzheimer's disease mouse model. *Biochem. Biophys. Res. Commun.* 2009; **379**, 406–410.

Escribano et al. (2010). Rosiglitazone rescues memory impairment in Alzheimer's transgenic mice: mechanisms involving a reduced amyloid and tau pathology. *Neuropsychopharmacology* 2010; **35**, 1593–1604.

Rodriguez-Rivera et al. Rosiglitazone reversal of Tg2576 cognitive deficits is independent of peripheral gluco-regulatory status. *Behav. Brain Res.* 2011; **216**, 255–261.

Pioglitazone Papers:

Masciopinto et al. Effects of long-term treatment with pioglitazone on cognition and glucose metabolism of PS1-KI, 3xTg-AD, and wild-type mice. *Cell Death Dis.* 2012; **3**, e448.

Searcy et al. Long-term pioglitazone treatment improves learning and attenuates pathological markers in a mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* 2012; **30**, 943–961.

Papadopoulos et al. Pioglitazone improves reversal learning and exerts mixed cerebrovascular effects in a mouse model of Alzheimer's Disease with combined amyloid β and cerebrovascular pathology. *PLoS One* 2013; **8**, e68612.

Mandrekar-Colucci et al. Mechanisms underlying the rapid peroxisome proliferator-activated receptor- γ -mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. *J. Neurosci.* 2012; **32**, 10117–28.

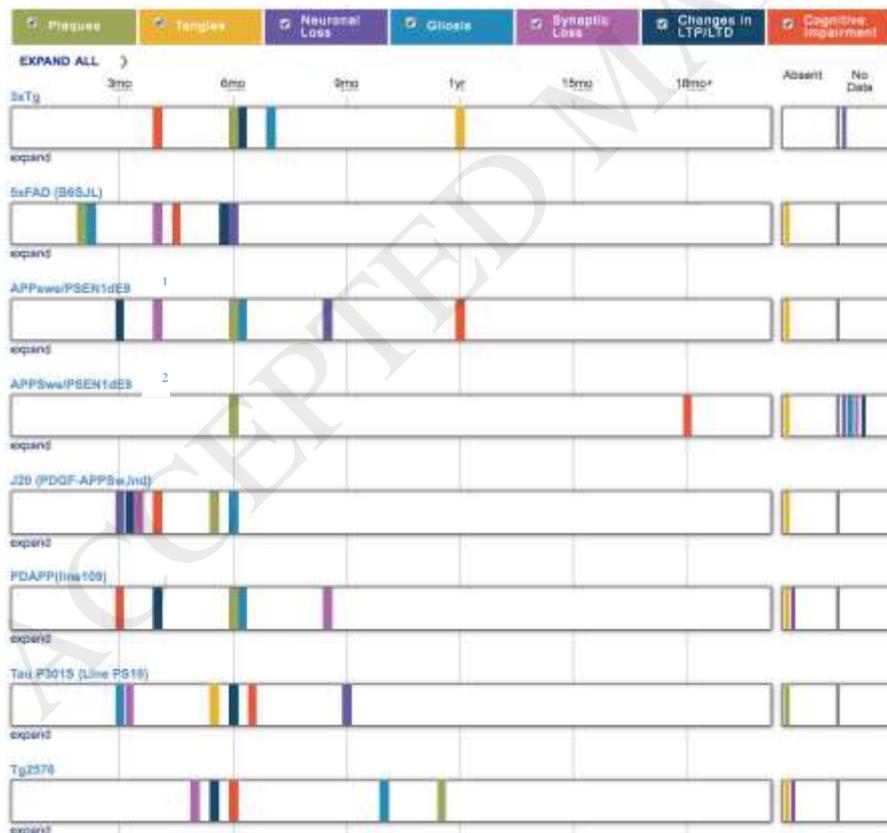
Nicolakakis et al. Complete rescue of cerebrovascular function in aged Alzheimer's disease transgenic mice by antioxidants and pioglitazone, a peroxisome proliferator-activated receptor gamma agonist. *J. Neurosci.* 2008; **28**, 9287–9296.

Chen et al. Anti-diabetes drug pioglitazone ameliorates synaptic defects in AD transgenic mice by inhibiting cyclin-dependent kinase-5 activity. *PLoS One* 2015; **10**, e0123864.

The quality of the remaining 15 papers were assessed for study design, mice characteristics and methodology, according to a specific set of criteria (10) (Appendix 1). Each paper was then scored according to this criteria (Table 2). These criteria were adapted from the ARRIVE guidelines, with the specific scoring for each criteria determined following further research and discussion.

A wide variety of genotypes were used in these papers, to produce models of AD in transgenic mice. As shown in Figure 2, each model develops different pathological features of AD at varying ages. 3xTg AD mice were found to be the most accurate representation of AD and thus scored 5 points. It is the sole model to incorporate 3 known AD specific genes, and develop both SPs and NFTs (11). The remaining models used single or double transgenic models that only demonstrated 1 of the pathological features of interest and thus scored poorly.

Figure 2. A visual model of AD pathology in certain models of AD in mice



A visual model comparing the different ages of onset (in months) of key pathology and symptoms of AD in the models of AD used in this thesis (ALZFORUM). Two models for

APP_{swe}/PSEN1dE9 are available. Whilst Mandrekar-Colucci et al. and O'Reilly and Lynch state that model 1 is used, Chen et al. and Matthes et al. do not clearly indicate which model was utilized, so may have used either.

This model was created on ALZFORUM's site and can be accessed at: (<http://www.alzforum.org/research-models/alzheimers-disease>)

mo – months old, yr – year.

The age and genotype of the mice used are closely interlinked, with the extent of the pathology differing depending on these 2 factors. Consequently, the age of the mice can considerably effect the outcomes. Excluding APP_{swe}/PS1dE9's delayed development of cognitive impairment (+12 months), the average age of onset (AAO) of cognitive decline was 4.67 months. Likewise, the average AAO for A β pathology was 5.83 months and tau was either at 5 or 12 months. Some studies introduced the drug at a far later age, potentially once the degree of pathology was too extensive for the drugs to effectively make any impact, and thus were scored poorly. Likewise, by starting the treatment before the average AAO of these pathological and cognitive features, experiments explored the potential for IS to be used as a preventative treatment rather than a cure, so received an equally low score.

The average duration of treatment was approximately 5 months. The longer the duration of the treatment, the more reliable the results, as this gave the drug more time to produce its effects. Thus, studies that administered the drug for more than 4 months, considerably increased the strength of their results and were scored highly.

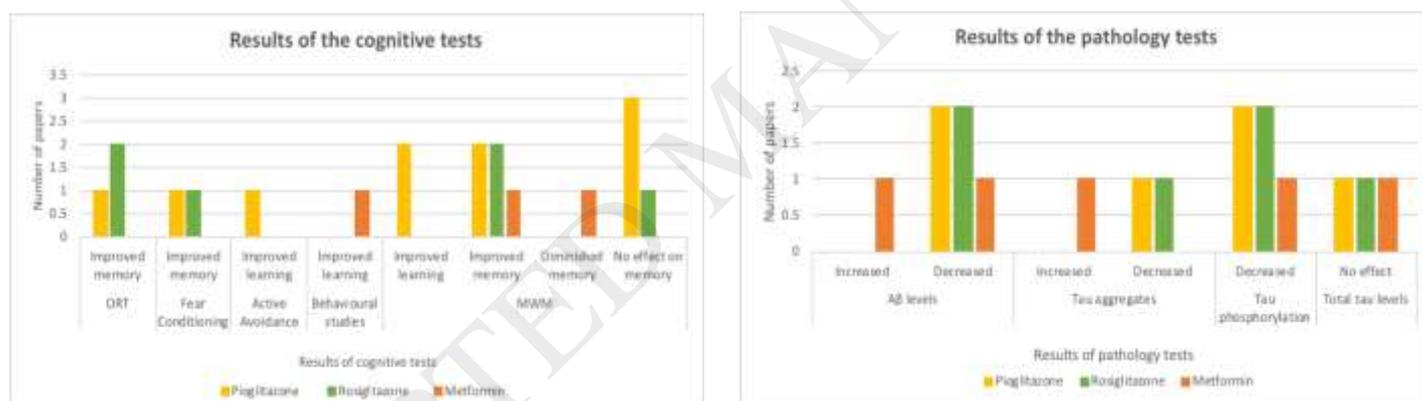
Table 2. The study quality of the included papers

	Author	Genotype	Age of mouse	Comparators	Sample size	Experimental and control groups	Treatment dose	Duration of treatment	Treatment Regimen	Baseline characteristics	Measures of cognitive function	Measures of characteristic pathology	Randomisation and blinding	Total
Rosiglitazone	Escriban et al. 2010	4	5	5	5	4	4	5	5	4	5	5	3	54
	Yu et al. 2015	5	4	3	5	5	5	5	4	5	5	4	0	50
	Rodriguez-Rivera et al. 2011	3	5	5	4	5	5	1	5	5	3	1	0	42
	Escriban et al. 2009	4	5	5	1	5	4	3	5	4	4	1	0	41
	O'Reilly & Lynch 2012	1	5	3	0	5	4	1	4	2	4	4	0	33
Pioglitazone	Mascioppo et al. 2012	5	2	4	5	5	4	4	5	4	5	1	4	48
	Searcy et	5	4	2	4	2	5	5	5	4	2	5	3	46

Results

Four out of five RTZ papers showed improved cognition and they all demonstrated improved A β pathology (Figure 3). In PTZ studies assessing both learning and memory, only learning was shown to improve. However, out of the four studies assessing purely memory, three identified some improvements in this area. Whilst two papers reported an improvement in A β pathology, two reported no effect. Only two MET papers evaluated cognition. Whilst one paper reported improved learning, the other only reported improved memory in females, with increased memory impairment seen in males. One paper reported an increase in A β pathology and another a decrease. All three drugs reported a decrease in tau phosphorylation, but no effect on total tau levels.

Figure 3. Graphs showing the results of the cognitive and pathology tests



Only 2 studies used more than one cognitive test and 2 papers used no cognitive tests at all. 8 papers measured A β levels and only 4 papers investigated tau pathology. MWM – Morris Water Maze, ORT – Object Recognition

Discussion

This paper aims to critically assess whether IS are effective at reducing the characteristic cognitive impairment and pathology seen in AD, by looking at models of AD in transgenic mice. Whilst the majority of the results present an aspect of cognitive improvement, the findings are heterogeneous, reducing confidence in the outcomes produced. Fewer papers assessed the effects on AD pathology, producing similarly conflicting evidence. The scarcity

of evidence, alongside the heterogeneous results, hinders the formation of any definitive conclusions.

Rosiglitazone

RTZ was found to be effective at improving cognition, A β pathology and tau phosphorylation, suggesting it is the most effective IS. Although the majority of the evidence supports this conclusion, it should be treated with caution, as systematic errors in the methodology resulted in poor internal validity and reliability.

Pioglitazone

PTZ had the highest internal validity, incorporating elements of randomization and blinding that were notably absent from the other drugs. However, flaws in the study design produced substantial heterogeneity within the results. PTZ has been shown to be effective at decreasing tau phosphorylation and improving learning, more than memory, in mice. Whilst the evidence in support of PTZ's ability to improve A β pathology is more reliable than the evidence against it, the results are still inconclusive, with the overall reliability of the data weak in support of either argument.

Metformin

The low quality of the study designs in every MET paper made it impossible to draw any definitive conclusions on its efficacy. Although it is found to be similarly effective at reducing tau phosphorylation, the reliability of the data on cognition and A β pathology is poor, hindering any conclusive outcomes from being determined.

Study quality

The heterogeneous nature of the results can be contributed to the numerous confounders and poor study design of the papers analyzed. A variety of cognitive tests and measures of pathology were utilized to assess these outcomes, hindering comparison. Similarly, mice characteristics varied hugely between papers. The papers exhibited numerous forms of bias, reducing the internal validity and impacting the reliability of the outcomes. Instances of selection, performance, detection and attrition bias have been identified in the papers

analyzed. This low internal validity is highlighted in Table 2, with insufficient blinding and a lack of randomization particularly prevalent. This increases bias and thus reduces the reliability of the results. Additionally, the use of mice significantly reduces the external validity of these studies as the ability to extrapolate these results into humans is limited by the biological differences between the two.

Current context

Previous studies investigating the use of TZD to improve cognition in mild-to-moderate AD patients have produced conflicting results. Early phase II (12) and phase III (13) double-blind, randomized, placebo-controlled trials showed improved cognition upon RTZ treatment. However, more recent phase III clinical trials (14,15) have found no effect of RTZ treatment on cognition. Similarly, smaller clinical trials for PTZ disagree on the efficacy of this drug in mild-to moderate AD patients. 2 pilot clinical trials showed improved memory and cognition in patients with mild AD and T2D (16,17), whereas no benefit was found in a similar study which used patients with AD but not T2D (18). This suggests that PTZ may only have an effect in patients with diabetes. There are currently no clinical trials on the use of MET in patients with AD. In order for IS to be successful in humans, how they exert their effects on mice has to be determined. The differing efficacy between TZD and MET suggest it can't be purely due to their effects on glucose regulation. Therefore, by determining what element in their mechanism of action improves cognition and pathology, treatment can target this factor and potentially be used more effectively in humans.

Conclusion

Initially the results from these studies appeared too heterogeneous to conclusively determine any effect by IS. However, analysis of the accuracy and reliability of the studies highlighted outcomes that supported the IS's, in particular the TZD's, ability to improve the symptoms and pathology of AD in mice. However, the level of confidence in these conclusions is significantly impacted by consistent flaws in the study design. In order to confidently conclude that IS are effective, more studies of greater internal validity are required. The disparity in the efficacy of each drug, suggests that it is not purely the insulin sensitizing effects that are inducing a response. Therefore, although this paper suggests a level of efficacy

in TZD at improving the symptoms and pathology of AD in mice, studies of a greater quality, accuracy and internal validity are needed to learn more about how IS exert their effect, before they can be effectively used in humans.

Declaration of Interest

The Authors declare no conflicts of interest in preparing this review.

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