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Original Article

Effect of metformin on cognitive dysfunction in animal with insulin resistance: a non-quantitative systematic review of randomized controlled trials

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Abstract: Background: Current reports from observational studies and randomized controlled trials (RCTs) on the effect of metformin on cognitive dysfunction are inconsistent. This systematic review aimed at analyzing the effect of metformin on cognitive and memory function in animal models with insulin resistance (IR). Methods: We conducted a literature search of PubMed, Embase, Sinomed, the Cochrane library, CNKI, Wanfang, VIP and clinical trial registries, for English and Chinese articles on RCTs exploring the association between metformin and cognitive dysfunction induced by IR in animals. Heterogeneity and paucity of original data precluded a meta-analysis, and hence, two independent reviewers performed a non-quantitative systematic review of RCTs. Results: Eight studies met the inclusion criteria; two were in English and others in Chinese. Two studies were in mice models and the rest in rat models. Coincidently, all of these studies reported that metformin had beneficial effects on cognitive function. Conclusions: Studies in animal models with IR suggest improvements in cognitive dysfunction by metformin. The results imply a possible therapeutic benefit of peripheral insulin-sensitizer metformin in AD and mild cognitive impairment (MCI). These findings also provide direct evidences linking IR and AD or MCI. However, further studies are required to confirm the effects of metformin on cognitive dysfunction.

Keywords: Cognition disorders, Alzheimer disease, diabetes mellitus, insulin resistance, metformin

Introduction

Globally, most individuals might be at an increased risk of dementia given the trends for increasing incidence of obesity, hyperinsulinemia, insulin resistance (IR) and diabetes mellitus (DM). Alzheimer's disease (AD), the most common cause of dementia, is characterized by progressive cognitive impairment, loss of memory and abnormal behavior. AD is very common in the elderly. Genetic and environmental factors contribute to its occurrence and development. Currently, DM is one of leading causes of increase in the incidence of disease. It is expressed as a set of metabolic syndrome characterized by hyperglycemia resulting from pancreatic island function defects or/and insulin resistance. Metformin is widely used as a first line drug in patients with type 2 DM for the reduction of hepatic glucose output and increase in the insulin-mediated utilization of glucose, and lowering of insulin levels. Previous studies demonstrated that metformin can rapidly cross the blood brain barrier and has several beneficial effects in the brain such as anti-inflammatory and neuroprotective effects.

DM and AD are both common chronic diseases that can increase the functional disability in patients and social burden. Epidemiological studies have shown an association between DM and an increased risk of mild cognitive impairment and dementia. More and more research groups have reported of an association between DM and AD. This relationship is so close that some authors have defined AD as Type 3 Diabetes [1]. Numerous studies have suggested that the incidence of AD is two times

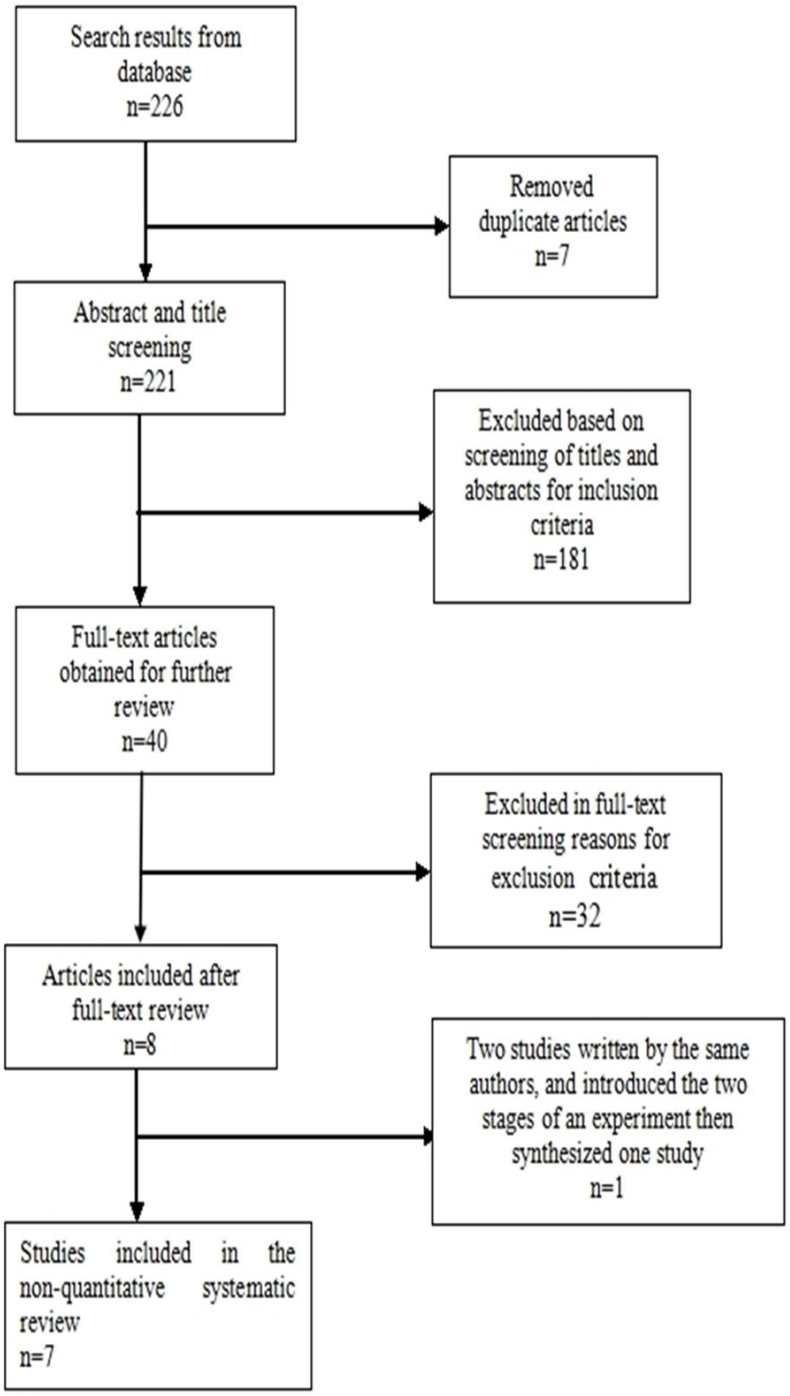

Figure 1. Literature search flow diagram.

higher in patients with DM than that of sporadic AD [2]. Rachel Whitmer, an American scholar, reported that the incidence of sporadic dementia is two times higher in patients with DM, and metformin significantly reduces the risk, while other anti-hyperglycemia agents (including insulin) increase the risk [3]. However, observational studies and randomized controlled trials (RCTs) have yielded inconsistent results on the effects of metformin on cognitive dysfunction. Heterogeneity and paucity of original data precluded a meta-analysis; hence, we proceeded with a non-quantitative systematic review of RCTs.

Methods

Search strategy

We conducted a search of the following databases: PubMed, Embase, Sinomed, the Cochrane library, CNKI, Wanfang, VIP and clinical registries, for RCTs on animal models exploring the association between metformin and cognitive dysfunction induced by IR. Two reviewers independently reviewed and analyzed the data. Any discrepancies were resolved by a consensus. Search terms used were (diabetic OR diabetes mellitus OR DM OR insulin resistance OR IR) AND (metformin OR MET OR dimethylbiguanidine OR dimethylguanylguanidine OR glucophage) AND (cognition OR Alzheimer's disease OR dementia OR amnestic OR AD OR MCI OR cognitive impairment OR memory impairment). We also hand searched the references of all eligible articles and related previous review articles (**Figure 1**). Studies were considered eligible if they fulfilled the following criteria:

Inclusion criteria

(1) they were published in English or Chinese language; (2) subjects were primary animals

wherein metformin was compared with a placebo or blank control; (3) subjects were primary animals with induced DM or IR; (4) they were RCTs; (5) the outcomes provided information on the change in cognition levels before and after metformin therapies in animals; (6) studies available as full-text article.

Exclusion criteria

(1) Population-based case-control studies or a single case reports, summary, conference reports; (2) metformin compared with insulin or other anti-hyperglycemic therapy oral drugs; (3) the experiment process described not detailed; (4) not induced DM or IR animal models.

Outcomes

The main outcome was the change in cognitive and memory function before and after metformin therapy. Cognition learning and memory was tested using water mazes, such as the Morris water maze, the Channel water maze, and the Barnes maze. Other key outcomes included: (1) change from baseline in plasma glucose and plasma insulin; (2) change from baseline in body weight and the threshold value for IR, the homeostatic model assessment-IR (HOMA-IR); (3) pathophysiology index change in the hippocampus tissue of animals.

Results

The initial electronic and manual searches yielded 226 potential literature citations, of which 186 were excluded after scanning the titles and abstracts. Two authors independently searched full articles for the remaining 40 citations, and excluded additional 32 studies for various reasons. Finally, eight RCTs that evaluated the effect of metformin on cognitive function in animals with IR were shortlisted. Of these, two studies were by the same authors; we opined that they are the two stages of an experiment, and therefore, considered it as one study. Among these eight studies, two studies were in English, and the rest in Chinese. Three studies were in mice models and the others in rat models. Coincidently, all of these studies observed that metformin had beneficial effects on brain neurons and effectively improved cognitive function. Since these studies introduced different mechanisms of action of metformin on cognition, and because only three studies had original data, a quantitative systematic review (meta-analysis) was not feasible. Hence, we proceeded with a non-quantitative systematic review of RCTs. The main characteristics of the eight trials are summarized in **Table 1**.

Discussion

AD is a complex and heterogeneous disorder and efforts to understand the pathogenesis of AD is ongoing; and hence, the role of DM in increasing the incidence of dementia is largely unknown. Long-term metformin treatment either improved or decreased cognitive function in animals with IR, as reported in the following studies.

Li et al. [4] reported that phospho-tau and the β-amyloid peptide (Aβ) levels were increased in the hippocampus area of mice with IR. Concurrently, the levels of phosphorylated/activated 54 KD c-Jun N-terminal kinase (JNK), a major tau protein kinase also significantly increased [5]. However, metformin attenuated the increase of total tau protein, phospho-tau, and activated JNK, and decrease of Aβ levels in the hippocampus of IR mice. This study suggested that metformin had a beneficial effect on cognition in IR animals.

Mo Wei et al. [6] put forth a new hypothesis that metformin improved peripheral IR, and protected the central nervous system. Research indicates that metformin can activate AMP-activated protein kinase (AMPK); AMPK plays an important role in cell lipid and glucose metabolism. AMPK affects learning and memory function [7]. Further, they suggested that metformin might improve learning and memory deficits in rats with IR by activating AMPK. They also observed that IR prevented insulin from entering the brain; thereby causing an aberration in the energy metabolism in the brain cells, leading to lactic acid accumulation and decline of Na^+-K^+-ATPase activation in these rats [6]. However, metformin could regulate energy metabolism by promoting the passage of insulin into the brain.

Yuan Xin et al. [8] reported that one of the characteristic pathological hallmark of AD is the presence of neurofibrillary tangles containing hyperphosphorylated tau, a microtubule-associated protein. IR in the brain causes excessive tau protein phosphorylation [9] that can lead to

Table 1. Characteristics of randomized controlled trials included in the non-quantitative systemic review

Author	Year	Animal Model	Methods of establish model	Number of Subjects (n)	Number of Controls (n)	Duration of Metformin	Dosage of Metformin	Route	Outcomes	Original Date Available	Result	Reference
Mo Wei	2014	180-200 g SPF male Wistar rats, 60 days	High-fat feed (30% carbohydrate, 12% protein, 52% fat, 6% other), continuous feed 12 weeks	15	15	4 weeks	Low dose (100 mg·kg^{-1}·day^{-1}) and high dose (200 mg·kg^{-1}·day^{-1})	Lavage	Plasma glucose, plasma insulin, HOMA-IR, body weight, visceral fat, total cholesterol, escape latency, swimming speed, time in quadrant in the Morris water maze study score, remember retention time, in the y-type electric maze, Na^+-K^+-ATPase activity, lactic acid content of the brain	Yes	Metformin may improve learning and memory deficits by activating AMPK	[6]
Yuan Xin	2014	160-180 g adult male SD rats	High-fat diet feed formula, continuous feed 4 weeks	10	10	4 weeks	2 mg/mL	Added metformin to drinking water	Plasma glucose, body weight, escape latency, swimming speed, the first time to reach the platform, the number of through the platform, time in quadrant in the Morris water maze	No	Metformin reduces insulin resistance and improved the animal's spatial cognition ability	[8]
Zhang Weihau	2011	18-22g, 4-week-old ICR mice	High sugar, high fat, high heat feed for four weeks, thereafter intraperitoneal injection STZ 100 mg/kg	10	10	3 weeks	250 mg/kg	Lavage	Escape latency and the number of errors in the channel water maze, change in hippocampal nerve cell pathology, number of TUNEL caspase-3 positive cells	Yes	Metformin improves learning and memory in mice with diabetes, and can protect hippocampal neurons	[19, 20]
Li Qiang	2012	200-250 g adult, male, SD rats	2% Streptozocin (25 mg/kg), continuous intraperitoneal injection for 3 days	12	12	5 weeks	55.33 mg·kg^{-1}·day^{-1}	Lavage	Blood glucose, NO content in the hippocampus	Yes	Metformin can lighten the toxicity of NO on neurons by decreasing NO content in the hippocampus, thus improving cognitive dysfunction during DM	[13]
Li Xianhui	2013	320±20 g 12-week male GK rats	Selected newborn male rats, 2 days after streptozocin intraperitoneal injection (90 mg/kg)	12	12	No more than 20 weeks	300 mg·kg^{-1}·day^{-1}	Added metformin to eating food	Plasma glucose, plasma insulin, HOMA-IR, body weight, escape latency, swim path and swimming distance, time in quadrant in the Morris water maze, hippocampal neuronal activity and the density of the dendritic spines	No	Metformin improves cognitive dysfunction in rats	[18]

Hiranya Pintana	2012	180-200 g, 5-week-old, male Wistar rats	59.28% fat, 14.27% carbohydrates, 26.45% protein with an energy content of 5.35 kcal/g and 59.28% fat of total energy	8	8	3 weeks	15 mg/kg, twice daily	Gavage	Body weight, plasma total cholesterol, total protein, plasma glucose, plasma insulin, HOMA-IR, plasma MDA, brain MDA, time to reach platform, time in target quadrant of the Morris water maze test, brain mitochondrial levels and oxidative stress levels	Incomplete	Metformin improved learning and memory behaviors in HFD-fed rats. Metformin attenuated brain mitochondrial dysfunction and decreased the brain oxidative stress levels in HFD-fed rats	[12]
Jiejie Li	2011	6-week-old male db/db mice	Diabetes-prone mice model	11	11	No more than 18 weeks	200 mg·kg^{-1}·day^{-1}	Intraperitoneal delivery of metformin	Lactate, glucose, plasma insulin, HbA1cbody weight, time spent in the hole, latency to find target box in the Barnes maze, expression of phosphorylated tau and total tau in the mouse hippocampus, ratio of phosphor tau to total tau, Aβ42 assay	Incomplete	Metformin attenuated the increase of phosphor-tau and of synaptophysin in the db/db mouse brain, suggesting a role in these animals with diabetes	[4]

Abbreviations: Aβ42, β-amyloid peptide; AMPK, AMP-activated protein kinase; HOMA-IR, homeostatic model of assessment-insulin resistance; SD, Sprague Dawley; MDA, methylenedioxyamphetamine; TUNEL, Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling; NO, Nitric Oxide; GK, Goto-Kakizaki; db/db, diabetes mouse; HFD, High-fat feed; ICR, Institute of Cancer Research; SPF, Specefic pathogen free.

entwining of nerve fibers [10] and skeletal damage to the nerve cells. IR can also promote Aβ deposition in the brain, thereby accelerating the symptomatic progress of AD and dementia [11]. However, metformin can rapidly cross the blood brain barrier and probably acts directly as a neuroprotective agent in the central nervous system. Thus, metformin aids in improving high blood sugar, reducing the formation of advanced glycosylation terminal product, improving blood flow to the brain and blood brain barrier function, and alleviating the extent of damage to the central nervous system caused by hyperglycemia [8].

Pintana et al. [12] demonstrated that oxidative stress in the brain and plasma, brain mitochondrial dysfunction, as well as the impairment of cognitive function could be induced by long-term high fat diet. Metformin improved the peripheral insulin sensitivity, thus decreasing plasma and brain mitochondrial oxidative stress, improved brain mitochondrial function, prevented mitochondrial membrane depolarization, and protected brain cells from mitochondrial swelling.

Li et al. [13] discovered that nitric oxide (NO), a kind of free gas molecule, played an important role in tumor destruction, immune defense, blood vessels balance, and nerve conduction. Diabetic microangiopathy could cause hippocampal ischemia, and subsequently increase the content of excitatory amino acid that could activate N-methyl-aspartate receptor, finally lead to excessive NO production [14, 15]. Excessive NO causes nerve toxicity [16, 17]. Metformin could aid in lowering NO toxicity by decreasing NO content in the hippocampus, thereby, leading to improve cognitive function in diabetes rats.

Li Xian-Hui et al. [18] indicated that cognitive dysfunction induced by DM may be due to the decline in the activity of protein kinase B or Akt of hippocampal neurons, which causes a reduction in cAMP-responsive element-binding protein (CREB) phosphorylation, which in turn, affect the transcription of a variety of target genes related to memory, eventually lead to learning and memory dysfunctions. Therefore, improvement in Akt/CREB signal pathway may be effective in treating diabetic encephalopathy. Metformin increases activity of neurons and the density of dendritic spines in the hippocampus. After intervention with metformin, the hippocampal neurons arranged closely, and the cell viability significantly increased, and there was a corresponding significant increase in the density of the dendritic spines. Metformin significantly activated the Akt/CREB pathway and increased the expression of memory-related proteins in the downstream part of the Akt/CREB pathway, such as Akt, CREB, synaptophysin and glutamate receptor 1. These results suggested that metformin activates these points to play a role in improving cognitive dysfunction.

Conclusion

In conclusion, we opine that these studies indicate that metformin ameliorates cognitive dysfunction in IR animal models receiving long-term metformin therapy. In this regard, our data indirectly provide the possibility that metformin might improve some of outcomes associated with AD and provide the basis for a therapeutic strategy for the human nervous system. But Clinical trials and further investigations are necessary to confirm the role of metformin on cognitive function in DM.

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Disclosure of conflict of interest

None.

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