Effect of Pioglitazone on Expression of Interleukin-17A, Toll-like receptor 2, Inducible nitric oxide synthase 2 and Arginase 1 in Alzheimeric-model of rats Brain.

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Abstract

Introduction: Neuroinflammation is actively involved in neurological disorders such as Alzheimer's disease and it is one of the primary and permanent features of Alzheimer's disease. PPAR γ receptors are widely distributed in the brain and it is very important for learning. Agonists of this receptor such as pioglitazone can have beneficial effects in the treatment of Alzheimer's disease. In this study, the effect of pioglitazone (PPAR γ pathway agonist) on gene expression of Toll-like receptor 2, interleukin A, inducible nitric oxide synthase 2, and arginase-1 in the hippocampus Alzheimer's model of male rats was investigated.

Methods: In this study, 35 male Wistar rats were randomly divided into five groups of seven. The groups include control, Alzheimer's Sham group scopolamine (3 mg/ kg) +DMSO, and three groups receiving scopolamine+ pioglitazone with doses 5, 10, and 20 mg/kg for 12 days. All injections are intraperitoneal. In the end, hippocampal tissue was extracted to evaluate the expression of the desired genes by Real-time PCR and finally, SPSS software was used for statistical analysis of data.

Results: It was observed that the gene expression of Toll-like receptor 2, interleukin A and inducible nitric oxide synthase 2 in the Sco+DMSO group increased compared to the control group (except in the arginase-1 gene) and also the gene expression of these factors in the groups receiving pioglitazone decreased compared to the Sco+DMSO (except in the arginase-1 gene).

Conclusion: Pioglitazone can decrease the gene expression of Toll-like receptor 2, interleukin A and inducible nitric oxide synthase 2 and increase the gene expression of arginase-1 in the rat brain. So pioglitazone is useful for the reduction of inflammation in the brain.

Background

The quality of life in Alzheimer's patients is low and often current therapy regimens are not sufficient to treat the disease. More than 70% of dementia cases are Alzheimer's disease (AD), and in the 1990s, approximately 20.2 million cases of AD were reported. At the end of 2020, about 50 million people were reported to have AD and by the end of 2050, that number is expected to may reach 125 million [1].

Pathologically, AD is marked by the presence of amyloid β (A β) deposition and neurofibrillary tangles, the cholinergic system dysfunction, increase of oxidative stress, activation of microglia and, steady neuroinflammation [1]. Inflammatory markers detection in AD shows a potential role for neuroinflammation in the pathogenesis of this disease [2]. Neuroinflammation, the major checkpoint in AD's pathogenesis, is marked by an increase in proinflammatory cytokines, immune cells infiltration, and activated glial cells [3]. One of the main causes of neuroinflammation in AD is microglial activation [1] which is associated with the secretion of inflammatory cytokines and chemokines [4]. Microglia produces interleukin-17 (IL-17) protein [5] and this cell expresses toll-like receptors [6], inducible nitric oxide synthase (iNOS) [7]. Additionally, arginase 1 is another microglial activation marker that competes with iNOS for L-arginine and inhibits nitric oxide production [8].

Microglia is the first line of defense versus pathogens and other brain tissue injuries [9]. Microglia express types of toll-like receptors such as toll-like receptor 2 (TLR2) in the brain [6] which is a member of pattern-recognition receptors in the innate immune system [10]. Toll-like receptors play a critical role in AD [6] and the gene of TLR2 is highly expressed in AD in the brain areas containing plaque formation [9]. This receptor has a dual role in AD [9] including the clearance of A β accumulation [11, 12], and induction of expression of pro-inflammatory molecules in response to A β [11]. Indeed, stimulation of TLR2 by activating microglia induces A β phagocytosis [9], and also, upregulating this receptor may be a treatment option in AD by regulating the phagocytic activity of microglia [13].

IL-17, a pro-inflammatory cytokine [14], acts as a central regulator of the proinflammatory reaction in the brain [5, 15]. IL-17 mRNA expresses in astrocytes and oligodendrocytes and IL-17 protein produces in microglia [5]. In AD, IL-17 is produced from microglia and its expression is increased in the hippocampus, cerebrospinal fluid, and serum after A β -42 peptide injection. But the exact mechanism of upregulation of IL-17 in the brains of AD patients is still unknown [16].

iNOS express in central nervous system cells such as neurons and microglia [7, 17] and is involved in microglial activation [17]. In vivo and in vitro studies demonstrated that iNOS can induce neuronal apoptosis and also induce apoptosis in macrophages, astrocytes, and differentiated PC12 cells. Thus, neuronal damage in AD can reduce by inhibition of neuronal iNOS expression [18].

Hydrolysis of L-arginine to L-ornithine and urea is performed by arginase, which is a manganese metalloenzyme [19]. This enzyme is associated with central nervous system disorders including AD, multiple sclerosis, diabetic retinopathy, and retinopathy of prematurity. Two different genes encode two isoforms of arginase including arginase 1 and arginase 2 [20]. Brain-infiltrating macrophages on microglia express arginase 1 after central nervous system damage and these macrophages rely on arginase 1 expression to indicate a repair role to ameliorate injuries [21]. In an AD mouse model, IL-4 injection was induced arginase 1+ microglia expression, which remarkably reduced A β plaque accumulation under an IL-1 β dependent neuroinflammation [22].

To treat hyperglycemia in type 2 diabetes is used pioglitazone which is an agonist of the peroxisome proliferator-activated receptor (PPAR) [23, 24]. PPAR is a ligand-activated transcription factor [25] which have three types including α , δ/β , and γ [26]. These receptors have anti-inflammatory and antioxidant effects in brain damage or in neurodegenerative disorders including AD, ischemic stroke, and Parkinson's diseases [25]. PPAR γ express at the neuronal level in the rat hippocampus and cortex [27] and could be a new therapeutic approach for the treatment of AD [28]. Additionally, these receptors with expression in microglia and astrocytes exert anti-inflammatory effects in the central nervous system [29]. The activation of these receptors in some cells, including macrophages, monocytes, and microglial cells reduces proinflammatory cytokine and iNOS expression [18]. It is reported that pioglitazone activates the PPAR- γ receptor and has a protective effect versus neuronal degeneration and cognitive deficits induced by binge alcohol with inhibition of pro-inflammatory cytokines in rats [30].

Pioglitazone, a thiazolidinedione derivative, inhibits Pam3CSK4-induced TLR2 expression in human monocytes and db/db mice which indicates anti-inflammatory effects of this drug [31]. Also, the level of inflammatory bone destruction decreased with pioglitazone and this drug decrease the circulating and local expression of IL-17 in rats with adjuvant-induced arthritis [32].

Scopolamine, a non-selective muscarinic cholinergic antagonist, impairs memory and cognitive functions in human and animals [33], and this drug increase pro-inflammatory cytokines, and inflammation [33, 34]. Scopolamine may induce neuroinflammation by increasing the level of oxidative stress and proinflammatory cytokines in the hippocampus [35].

Although there are many studies on PPAR γ and pioglitazone, there are few studies on the effect of pioglitazone on anti-inflammatory and pro-inflammatory factors in the AD model of rats. In the current study, we used a scopolamine-induced model of AD to show the treatment effect of pioglitazone on neuroinflammation and to provide a feasible theoretical basis and therapeutic target for AD treatment.

Methods

Animals

Adult male Wistar rats at 10 weeks of age were obtained from Pishro mehravaran azma pars (Babul, Iran), kept individually, and had access to free food and water. Rats acclimatized for 1 week in an animal room at 22 $\pm$ 3 °C temperature with a 12/12-h light-dark cycle. All experimental methods were performed in accordance with the National Institutes of Health Guide (NIH Publications No. 8023, revised 1978) for the Care and Use of Laboratory Animals. This study was approved by the Ethics Committee of Golestan University of Medical Sciences, Gorgan, Iran (Ethics number: ir.goums.rec.1399.346).

Experimental design

A total of 35 Wistar rats were randomly divided into 5 groups; each group included 7 rats, as follows:

Group 1: A control group that did not receive any drug.

Group 2 (Sco+DMSO group): A group that received scopolamine at a dose of 3 mg/kg/day [36, 37] for one day and DMSO injection for 12 consecutive days.

Group 3 (Sco+Pio 5 mg/kg group): A group that received scopolamine at a dose of 3 mg/kg/day for one day and was treated with pioglitazone 5 mg/kg/day for 12 consecutive days.

Group 4 (Sco+Pio 10 mg/kg group): A group that received scopolamine at a dose of 3 mg/kg/day for one day and was treated with pioglitazone 10 mg/kg/day for 12 consecutive days.

Group 5 (Sco+Pio 20 mg/kg group): A group that received scopolamine at a dose of 3 mg/kg/day for one day and was treated with pioglitazone 20 mg/kg/day for 12 consecutive days.

All injections were intraperitoneal and pioglitazone was dissolved in DMSO.

48 hours after the last drug injection and after sacrificed rats, the hippocampus was rapidly isolated from rat brain tissue and used to study gene expression by real-time polymerase chain reaction. (RT-PCR) method.

RNA extraction and RT-PCR

The mRNA expression of IL-17A, TLR2, iNOS2, and arginase 1 were specified with the RT-PCR method. Total RNA was extracted from hippocampus tissue with the use of the Pars Tous RNA isolation Kit (Mashhad, Iran) according to the manufacturer's protocols. Their concentration was measured using NanoDrop. cDNA was synthesized using the cDNA synthesis kit (Pars Tous, Mashhad, Iran) following the manufacturer's protocols. The following specific primers were used: GAPDH Forward: TGCCACTCAGAAGACTGTGG and GAPDH Reverse: TTCAGCTCTGGGATGACCTTG; Arginase-1 Forward: AAGAAAAGGCCGATTCAC and arginase 1 Reverse: CACCTCCTCTGCTGTCTTC; iNos2 Forward: ACCACCCTCCTCGTTTCAG and iNOS2 Reverse: CAATCCACAACCTCGCTCCA; TLR2 Forward: CGCTTCCTGAATTTGTCC and TLR2 Reverse: GTTGTCGCCTGCTTCCA; IL-17A Forward: CTACCTCAACCGTTCCAC and IL-17A Reverse: TCTCAGGCTCCCTCTTCAG. Finally, the $2^{-\Delta\Delta CT}$ method was used as a relative quantification method to characterize the mRNA level of each gene [38].

Statistical analysis

Data were reported as the mean $\pm$ SD. Data were statistically analyzed by ANOVA using the SPSS software version 16.0 (Armonk, USA). Differences among means of multiple groups were compared with Tukey's test, and $P < 0.05$ was statistically significant.

Results

Effect of pioglitazone on the mRNA expression of IL-17A

Administration of scopolamine to rats increased significantly the mRNA expression of IL-17A in the Sco+DMSO group compared with the control group (Fig. 1, $P < 0.05$). Injection of pioglitazone (5, 10, and 20 mg/kg) to rats decreased significantly the mRNA expression of IL-17A compared with the Sco+DMSO group (Fig. 1, $P < 0.05$). Effective dose was 20 mg/kg pioglitazone.

Effect of pioglitazone on the mRNA expression of TLR2

mRNA expression of TLR2 increased significantly in the Sco+DMSO group compared with a control group (Fig. 2, $P < 0.05$). Treatment of rats with different doses of pioglitazone (5, 10, and 20 mg/kg) decreased mRNA expression of TLR2 (Fig. 2). But this reduction was not significant compared with the Sco+DMSO group (Fig. 2) and also this reduction in mRNA expression of TLR2 was significant compared with the control group (Fig. 2, $P < 0.05$).

Effect of pioglitazone on the mRNA expression of iNOS2

Scopolamine administration could increase the mRNA expression of iNOS2 in the Sco+DMSO group compared with the control group (Fig. 3, $P < 0.05$). Pioglitazone could decrease the mRNA expression of iNOS2 compared with the Sco+DMSO group (Fig. 3, $P < 0.05$). Also, this reduction in the mRNA expression of iNOS2 was significant compared with a control group (Fig. 3, $P < 0.05$).

Effect of pioglitazone on the mRNA expression of arginase 1

The mRNA expression of arginase 1 reduced significantly with scopolamine injection in Sco+DMSO group compared with control group (Fig. 4, $P < 0.05$). Pioglitazone Injection (5, 10 and 20 mg/kg) to rats increased significantly the mRNA expression of arginase 1 compared with Sco+DMSO group (Fig. 4, $P < 0.05$). Effective dose was 20 mg/kg pioglitazone.

Discussion

Our results demonstrated that the treatment of pioglitazone, which is one of the PPAR γ agonists, led to a decrease in the mRNA expression of IL-17A, TLR2, and iNOS2 and an increase in the mRNA expression of arginase 1 in the rat hippocampus.

Important factors for induction/stimulation of AD are pro-inflammatory cytokines [39] such as IL-17A [40]. IL-17A, a signature cytokine of T helper 17 cells, is involved in inflammation [41] and may have an important role in AD pathogenesis [41, 42]. Behairi et al. (2015) and Hamdan et al. (2014) showed baseline levels of IL-17A and the serum level IL-17A were significantly higher in AD patients compared with controls [40, 42, 43]. Also, Liu et al, (2021) [44] reported an increase in IL-17 in the hippocampus of APP/PS1 transgenic mice, and IL-17 induced an increase of A β levels in mice. We also found that mRNA expression IL-17A increased in the hippocampus of scopolamine-treated rats.

Intrahippocampal injection of A β 42 into rats increased the expression of IL-17A in the hippocampus and also increased the cerebrospinal fluid and the serum of IL-17A in AD occurrence and development [45]. One of the plasma biomarkers for diagnosis of AD and neocortical A β load is the plasma IL-17A levels [46, 47]. These findings suggest that blockage of IL-17 may reduce A β -induced neurotoxicity and cognitive impairment in AD [44].

Nishimori et al. found that in response to bacterial entry into the intestinal mucosa, they produced IL-17A by direct TLR2 activation [48]. It is reported that energy deprivation up-regulated TLR2 in neurons and this may play a role in the AD pathogenesis [49]. TLR signaling have both beneficial and deleterious in AD and TLR2 expression can increase in the temporal cortex in AD [50, 51]. Also, TLR2 up-regulated markedly compared to the control in a transgenic mouse model of AD [52]. It is reported that TLR2 deficiency decreased A β deposition and neuroinflammation in a mouse model of AD [53]. Also, TLR2 deficiency decreased A β -induced inflammation, increased A β clearance [54] in cultured microglia, and ameliorated tauopathies in mice, which shows useful effects of TLR2 in AD [55, 56]. Additionally, we found that mRNA expression TLR2 increased in the hippocampus of AD model rats.

iNOS expression can upregulate with IL17A, which is a proinflammatory cytokine [57]. iNOS appears to be a major initiator of A β deposition and AD progression, thus a treatment option for AD may be inhibition of iNOS [58]. It is reported that intracerebroventricular A β 1–40 injection increase iNOS expression. The iNOS inhibition or the iNOS genetic deletion significantly ameliorates A β 1–40-induced impairment of learning and memory. The expression of iNOS has been strongly linked with AD pathology [59]. As we showed in this study, injection of scopolamine into rats to model AD increased the mRNA expression iNOS in the rat hippocampus.

In humans, arginase 1 is a cytosolic enzyme expressed in various areas of the brain especially in hippocampal neurons [19]. Arginase 1 is a marker of inflammation and stable arginase 1 expression reduces tau pathology and inflammatory response [60]. This enzyme has an important role in AD pathogenesis [19] so Kan et al., found that arginase 1 is extremely expressed in A β deposition areas [61]. It is reported that arginase 1 insufficiency in lysozyme M increased A β deposition, activated microglia, and behavioral impairments [21]. In this research we showed that scopolamine could decrease the mRNA expression arginase 1 in the rat hippocampus.

Inflammatory responses can modulate with PPAR γ agonists in the brain [18]. The PPAR γ agonist such as pioglitazone is FDA approved drug for diabetes treatment and has been used for treatment aims in animal models of central nervous system damage [62]. It is reported that the administration of pioglitazone improved inflammatory markers by reducing tumor necrotic factor α levels [63]. Also, it found that pioglitazone has an anti-inflammatory effect in the animal models of sub-acute and chronic inflammations [64]. As well as, regulating the PPAR γ /NF- κ B pathway by pioglitazone nanoliposomes reduce the inflammatory response in sepsis-induced acute lung injury [65]. Also, pioglitazone administration reduces inflammation following traumatic brain injury [62]. Also, we found that in this study, pioglitazone can decrease inflammation in rat hippocampus after scopolamine injection.

One of the ways in which pioglitazone (1 μ M) can induce an anti-inflammatory effect is by inhibiting the Pam3CSK4-induced TLR2 expression in human monocytes and db/db mice [31]. Also, we found that pioglitazone (5, 10, and 20 mg/kg) can decrease the mRNA expression of TLR2 in scopolamine-treated rats.

Pioglitazone (20 mg/kg, p.o. daily for 14 days), can inhibit the iNOS expression in mice model of AD [66]. In another study, pioglitazone could inhibit iNOS production in lipopolysaccharide-stimulated microglial cells [67]. As we found in this study, treatment with pioglitazone at different doses (5, 10, and 20 mg/kg, for 12 days) decreased the mRNA expression iNOS in rat hippocampus. It is reported that pioglitazone could decrease iNOS expression and increase the arginase 1 expression [68, 69]. An in vivo study showed that expression of arginase 1 can increase in aortic macrophages isolated from pioglitazone-nanoparticle-treated mice [70]. As well, we showed that treatment with pioglitazone increases the mRNA expression of arginase 1 in scopolamine-treated rats.

These studies further suggest a potential benefit of pioglitazone in managing neuroinflammation, with reduction of pre-inflammation genes expression and increase of anti-inflammation genes expression.

Conclusion

It can be concluded that Pioglitazone with decreases the gene expression of Toll-like receptor 2, interleukin A and inducible nitric oxide synthase 2 and with increases the gene expression of arginase-1 in the rat brain, can reduce the inflammation in the rat's brain. So, Pioglitazone is useful for the protection of the brain against inflammation.

Abbreviations

AD: Alzheimer's disease; A β : amyloid β ; IL-17: interleukin-17; iNOS: inducible nitric oxide synthase; TLR2: toll-like receptor 2; PPAR: peroxisome proliferator activated receptor; DMSO: Dimethyl sulfoxide; RT-PCR: real-time polymerase chain reaction.

Declarations

Ethics approval

This study was approved by the Ethics Committee of Golestan University of Medical Sciences, Gorgan, Iran (Ethics number: ir.goums.rec.1399.346).

Consent for publication

Not applicable.

Availability of data and materials

You can request data information via the email below:

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Competing interests

The authors confirm that there are no competing interests to declare.

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Authors' contributions

Parisa Rahimi Chari collected the study data and writing the manuscript. Mehrdad Jahanshahi participated in hypothesis formation, supervision of data analysis, and writing the manuscript. Ali Memarian and Leila Elyasi analyzed the data and participated in writing the manuscript; all authors participated in critical revision of the manuscript and approved the final version.

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Figures

Figure 1

Effect of different doses of pioglitazone (5, 10 and 20 mg/kg) on the mRNA expression of IL-17A in rat hippocampus. * means $P < 0.05$.

Figure 2

Effect of different doses of pioglitazone (5, 10 and 20 mg/kg) on the mRNA expression of TLR2 in rat hippocampus. * means $P < 0.05$.

Figure 3

Effect of different doses of pioglitazone (5, 10 and 20 mg/kg) on the mRNA expression of iNOS2 in rat hippocampus. * means $P < 0.05$.

Figure 4

Effect of different doses of pioglitazone (5, 10 and 20 mg/kg) on the mRNA expression of arginase 1 in rat hippocampus. * means $P < 0.05$.