Probiotics improve insulin resistance status in an experimental model of Alzheimer’s disease

Somayeh Athari Nik Azm1, Abolghassem Djazayeri1, Majid Safa2, Kian Azami3, Mahmoud Djalali4, Mohammad Sharifzadeh4*, Mohammadreza Vafa5**

Received: 23 Mar 2017 Published: 18 Dec 2017

Abstract

Background: Nowadays, Alzheimer’s disease (AD) is considered as Type 3 diabetes in which insulin resistance is the common cause of both diseases. Disruption of insulin signaling cascade and insulin resistance can induce AD; and central insulin resistance causes systemic alterations in serum insulin, FBS levels, and lipid profile. Studies have shown that probiotics (Lactobacillus and Bifidobacterium species) can be used as a nutritional approach to improve these metabolic changes. We assessed the probiotic effect (4 species of Lactobacillus and Bifidobacterium) on insulin resistance biomarkers in an experimental model of AD.

Methods: A total of 60 rats were divided into 5 groups: (1) a control group without surgical and dietary intervention; (2) a control-probiotics group receiving probiotics for 8 weeks, but not receiving any surgical intervention; (3) a group receiving a sham operation in which PBS was injected intrahippocampus but without dietary intervention; (4) an Alzheimer group for which Amyloid-β (Aβ) 1-42 was injected intrahippocampus but without dietary intervention; and (5) an Alzheimer-probiotics group for which Aβ1-42 was injected intrahippocampus and given 2g probiotics for 8 weeks. The FBS levels and lipid profile were measured by a calorimetric method, insulin levels were detected by an ELISA kit, and HOMA-IR was calculated using a formula. ANOVA (one way analysis of variance followed by Bonferroni comparisons post hoc) was used to compare all the variables between groups.

Results: Serum glucose, insulin levels, and HOMA-IR index increased in the Alzheimer group compared to the control (p<0.001), while probiotics decreased only insulin level and HOMA-IR index in AP group compared to Alzheimer group (p<0.001). Also, TG levels increased in the Alzheimer group (p<0.001), but no significant difference was detected between Alzheimer and Alzheimer-probiotics group.

Conclusion: It seems that probiotics play an effective role in controlling glycemic status of Alzheimer’s disease.

Keywords: Alzheimer Disease, Lactobacillus, Bifidobacterium, Insulin, HOMA

Introduction

Alzheimer’s disease (AD), as a neurodegenerative disease, causes impaired cognition function and memory loss (Tillisch et al. 2013). Nowadays, the number of patients with AD is estimated to be 36 million worldwide, and it is estimated that the number will be quadrupled in 2050 (Ríos et al. 2014, Saxena et al. 2011). Although less than 5% of AD cases are the inherited familial type, the sporadic type (late-onset) is the most common type of AD, and environmental factors affect its prevalence (Saxena et al. 2011). Observations have revealed that amyloid cascades, oxidative stress, neuroinflammation, insulin resistance, and apoptosis are involved in AD pathogenesis; nevertheless, the exact mechanism of AD is not known (Li et al. 2010).

Amyloid-β (Aβ) Aβ (1-42), produced by Amyloid protein precursor (APP), has a neurotoxicity effect and is
Probiotics effect on insulin resistance

In this study, 60 male Wistar rats (weight: 180-220 g, 8 weeks) were purchased from the Faculty of Pharmacy at Tehran University of Medical Sciences. Each rat was housed in one cage and all cages were kept at 25±2 °C temperature and on a 12-hour light/dark cycle. All the rats had access to ad libitum food (chow) and water. Our experiments were approved by the ethical committee for the care and use of laboratory animals at Tehran University of Medical Sciences (code: 902-1324001) and council of graduated students of Iran University of Medical Sciences (code: 93-01-27-24403).

Rats were divided into 5 groups: (1) a control group (C) without surgical and dietary interventions; (2) a control-probiotics (CP) group that received probiotics in drinking tap water for 8 weeks but received no surgical intervention; (3) a group receiving a sham operation (S) for which phosphate buffered saline (PBS), as the solvent of Aβ, was injected intrahippocampus, but without any dietary intervention; (4) an Alzheimer group (Aβ) for which 50 ng/µL side β-amyloid was injected intrahippocampus, but without dietary intervention; and (5) an Alzheimer-probiotics (AP) group that received 2 g probiotics with drinking tap water during 8 weeks (with 4 weeks interval before and after Aβ injection) in addition to surgical intervention (Aβ injection).

Beta-amyloid (1-42) preparation
Aβ (1-42) (sigma-Aldrich, USA) was dissolved in PBS (the solvent of Aβ). The solution was incubated for 5 days at 37°C and diluted by PBS to 50 ng/µL concentration on the test day. (Aβ dosage was selected based on previous studies (Asadi et al. 2015, Eftekharzadeh et al. 2012, Shariatpanahi et al. 2015) and our pilot study).

Probiotics preparation
Powdered preparation probiotics was obtained from Tak-Gen Company (Tehran, Iran). Based on the results of our pilot study, water volume was calculated (30 mL) and 2 g of probiotics (probiotics dosage was selected based on a previous study (Davari et al. 2013) and our pilot study) including lactobacillus acidophilus, fermentum, Bifidobacterium lactis, and longum (10^10 colony forming units (CFU)/g) were dissolved in an amount of calculated drinking tap water every morning. Water intake was monitored in all groups. Probiotics were selected based on antioxidative and anti-inflammatory effects, which have been reported in the past studies (Fijan. 2014, Hamaji et al. 2007, Messaoudi et al. 2011, Pang et al. 2012). Probiotics powder was colorless and odorless; at first, probiotics are inactive in water due to their special formulation, which includes a carrier compound that bonds to probiotics and allows probiotics activation in gut. Thus, the environmental factors do not affect probiotics in water.

Surgery procedure
Rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and Xylazine (5 mg/kg) and were

Methods

Study Design
In this study, 60 male Wistar rats (weight: 180-220 g, 8 weeks) were purchased from the Faculty of Pharmacy at Tehran University of Medical Sciences. Each rat was housed in one cage and all cages were kept at 25±2 °C temperature and on a 12-hour light/dark cycle. All the rats had access to ad libitum food (chow) and water. Our experiments were approved by the ethical committee for the care and use of laboratory animals at Tehran University of Medical Sciences (code: 902-1324001) and council of graduated students of Iran University of Medical Sciences (code: 93-01-27-24403).

Rats were divided into 5 groups: (1) a control group (C) without surgical and dietary interventions; (2) a control-probiotics (CP) group that received probiotics in drinking tap water for 8 weeks but received no surgical intervention; (3) a group receiving a sham operation (S) for which phosphate buffered saline (PBS), as the solvent of Aβ, was injected intrahippocampus, but without any dietary intervention; (4) an Alzheimer group (Aβ) for which 50 ng/µL side β-amyloid was injected intrahippocampus, but without dietary intervention; and (5) an Alzheimer-probiotics (AP) group that received 2 g probiotics with drinking tap water during 8 weeks (with 4 weeks interval before and after Aβ injection) in addition to surgical intervention (Aβ injection).

Beta-amyloid (1-42) preparation
Aβ (1-42) (sigma-Aldrich, USA) was dissolved in PBS (the solvent of Aβ). The solution was incubated for 5 days at 37°C and diluted by PBS to 50 ng/µL concentration on the test day. (Aβ dosage was selected based on previous studies (Asadi et al. 2015, Eftekharzadeh et al. 2012, Shariatpanahi et al. 2015) and our pilot study).

Probiotics preparation
Powdered preparation probiotics was obtained from Tak-Gen Company (Tehran, Iran). Based on the results of our pilot study, water volume was calculated (30 mL) and 2 g of probiotics (probiotics dosage was selected based on a previous study (Davari et al. 2013) and our pilot study) including lactobacillus acidophilus, fermentum, Bifidobacterium lactis, and longum (10^10 colony forming units (CFU)/g) were dissolved in an amount of calculated drinking tap water every morning. Water intake was monitored in all groups. Probiotics were selected based on antioxidative and anti-inflammatory effects, which have been reported in the past studies (Fijan. 2014, Hamaji et al. 2007, Messaoudi et al. 2011, Pang et al. 2012). Probiotics powder was colorless and odorless; at first, probiotics are inactive in water due to their special formulation, which includes a carrier compound that bonds to probiotics and allows probiotics activation in gut. Thus, the environmental factors do not affect probiotics in water.

Surgery procedure
Rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and Xylazine (5 mg/kg) and were
put in stereotaxic apparatus (Stoelting, wood Dale, IL, USA). For intrahippocampal injection of Aβ, using atlas of Paxinos and Watson (anterior-posterior, 3.08 mm; lateral, ± 2.2 mm; dorsal-ventral 2.7 mm from bregma), CA1 areas of hippocampus were detected, and injection was performed using a Hamilton syringe (50ng/µL/side). Every injection lasted for 1 minute; and to facilitate diffusion of Aβ, injection in the left side was performed after 60 seconds. Finally, the head of the rats was darned and they were returned to cages; probiotics intake began again in the next day.

**Glucose, Insulin levels, and Lipid profile detection**

After 12 hours of fasting, 5 mL blood was collected from all rats. The samples were kept for 30 minutes for clotting and were then centrifuged at 1500 rpm for 10 minutes. Clear serum was collected and kept in -80°C. Serum Glucose, triglyceride, total cholesterol, LDL, and HDL levels were detected by a calorimetric method with commercial kits (DiaLab GmbH, Austria). Insulin level was detected by ultrasensitive rat insulin ELISA kit (Mercodia, Sweden). Insulin resistance was determined using homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: fasting serum glucose (mg/dl) × fasting serum insulin (MU/dL) /405.

**Statistical analysis**

Data were analyzed using SPSS 23.0. Results were presented as Mean±SD. ANOVA (one way analysis of variance followed Bonferroni comparisons post hoc) was used to compare all the variables between groups.

**Results**

**Weight changes**

At baseline and at the end of the study, weight was not significantly different between groups. During the intervention, only weight showed a significant difference on the third week in AP group compared to C, CP, and S groups (Fig. 1).

**Insulin resistance biomarkers**

Figure 2 illustrates serum FBS levels. Aβ injection led to an increase in serum FBS level and indicated statistical difference in Aβ group (156.8±19.39) compared to C (113.17±9.94; P<0.001), S (115.67±12.68; p<0.001), and CP (127.75±26.32; p<0.001) groups. In the AP group, probiotics intake decreased serum FBS level (147.40±17.49) compared to Aβ group, but it was not significant, but it was higher than C, CP, and S (p<0.001) groups. Aβ injection led to a statistically remarkable increase in serum insulin level (1.46±0.21) (Fig. 3), and insulin resistance index was defined as HOMA-IR (0.56±0.11) (Fig. 4) in Aβ group compared to C (0.78±0.32 and 0.22±0.01), CP (0.79±0.29 and 0.25±0.12), and S (0.78±0.15 and 0.22±0.05) groups (p<0.001). In AP group, probiotics intake considerably reduced serum insulin level (0.93±0.14) and HOMA index (0.33±0.05) compared to Aβ group (p<0.001).
Probiotics effect on insulin resistance

The results of one study indicated that 80% of AD patients suffer from Type 2 diabetes or an impaired fasting glucose level (Burns et al. 2012). On the other hand, oxidative stress and inflammation observed in AD can affect systemic biomarkers. Various studies have found increased inflammatory and oxidative markers in the serum of AD patients. Inflammatory factors such as TNF-α lead to increased level of glucose and triglyceride production in the liver and decrease the peripheral glucose uptake, which causes insulin resistance (Mushtaq et al. 2015). Also, oxidative stress biomarkers can elevate FBS and HOMA-IR index (Salim et al. 2010). The elevated glucose level can affect the glucose uptake and may increase denevo lipogenesis and TG synthesis (Yadav et al. 2015). Consistent with the results of our study, many studies have suggested increased level of serum glucose, insulin, and HOMA in AD patients without diabetes (Burns et al. 2012, Li et al. 2015, Qiu and Folstein. 2006). Some studies have indicated that insulin resistance index (HOMA-IR) is mostly correlated with senile plaque and that AD patients are highly likely to develop diabetes. For example, the results of one study indicated that 80% of AD patients suffer from Type 2 diabetes or an impaired fasting glucose level (Kim and Feldman. 2015). It is possible that increased peripheral insulin level suggested in AD is compensatory for decreased insulin level in CNS and insulin signaling impairment. Also, results of some studies have suggested that pathological alterations of the brain related to AD commonly happen in regions regulating metabolism and satiety and could lead to systemic alterations of insulin level (Burns et al. 2012). Probiotics such as lactobacillus and bifidobacterium have beneficial effects on metabolic profile (Shimizu et al. 2015). Specific species of lactic acid bacteria such as L.acidophilus improves glucose intolerance, hyperglycemia, and hyperinsulinemia (Ruan et al. 2015). One Study shows that consumption of capsules containing L.acidophilus, L.casei, and B.bifidum decreases serum glucose level (Kim and Feldman. 2015). It is possible that increased peripheral insulin level suggested in AD is compensatory for decreased insulin level in CNS and insulin signaling impairment. Also, results of some studies have suggested that pathological alterations of the brain related to AD commonly happen in regions regulating metabolism and satiety and could lead to systemic alterations of insulin level (Burns et al. 2012). Probiotics such as lactobacillus and bifidobacterium have beneficial effects on metabolic profile (Shimizu et al. 2015). Specific species of lactic acid bacteria such as L.acidophilus improve glucose intolerance, hyperglycemia, and hyperinsulinemia (Ruan et al. 2015). One Study shows that consumption of capsules containing L.acidophilus, L.casei, and B.bifidum decreases serum

Table 1. The serum lipid profile biomarkers

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.5 ± 9.85</td>
<td>34.17 ± 5.81</td>
<td>27.08 ± 3.89</td>
</tr>
<tr>
<td>CP</td>
<td>75.33 ± 10.56</td>
<td>35.42 ± 5.29</td>
<td>27.08 ± 3.92</td>
</tr>
<tr>
<td>S</td>
<td>67.25 ± 8.58</td>
<td>33.92 ± 5.5</td>
<td>25.75 ± 4.20</td>
</tr>
<tr>
<td>Aβ</td>
<td>69.25 ± 9.21</td>
<td>32.67 ± 5.23</td>
<td>26.08 ± 3.96</td>
</tr>
<tr>
<td>AP</td>
<td>66.70 ± 6.5</td>
<td>30.10 ± 2.88</td>
<td>25.00 ± 3.05</td>
</tr>
</tbody>
</table>

The results are in Mean±SD. There was no significant difference between the groups (P>0.05). CP: control-probiotics; S: sham operation; Aβ: Alzheimer; AP: Alzheimer-probiotics.
insulin level and HOMA-IR, but it does not show any effect on FBS level and lipid profile in patients with major depressive disorder (Akkasheh et al. 2016). Another study suggests that intake of probiotics including *L.acidophilus*, *L.fermentum*, and *B.lactis* have resulted in a decreased level of FBS and insulin in STZ-induced diabetic rats (Davari et al. 2013). The results of a meta-analysis suggest that decreased level of glucose following probiotics intake is significantly higher in hyperglycemic objects. Interventions with duration over 8 weeks reveal a remarkable effect on FBS level; however, in interventions with durations of less than or equal to 8 weeks, a slight trend of glucose reduction have been observed. Also, amount of bacteria \( \geq 10^{11} \text{ CFU} \) is related to significant effects (Desbonnet et al. 2010). So, alteration in FBS, insulin levels, and HOMA-IR are confirmed in our study.

Also, probiotics cause PPARα (peroxisome proliferator-activated receptor) and CPT2 (carnitine-palmitoyltransferase) expression up-regulation, β-oxidation activation, and lipogenesis suppression that eventually lead to decreased TG level, as observed in our study (Park et al. 2013). Another meta-analysis demonstrated that probiotics significantly reduce the total cholesterol and LDL levels and they do not have any effect on HDL or TG levels. It is noted that when the baseline cholesterol and LDL levels are optimal, probiotics do not show any effects. The hypocholesterolemic effect of probiotics is stronger in trials over than or equal to 8 weeks. Also, bacterial strains and dosage are important factors (Cho and Kim. 2015). Since there were fewer baseline levels of cholesterol and LDL in our study, these parameters did not change.

Nevertheless, there were some limitations in our study. Given the costs associated with such undertaking, it was not possible to prolong the study. Also, it was better to study genetically modified mice as AD phenotype to avoid additional inconsistencies.

**Conclusion**

Overall, our study results indicate that probiotics can decrease insulin resistance markers such as serum insulin levels and HOMA-index, which have raised following intrahippocampal Aβ injection in an experimental model of Alzheimer’s disease.

**Acknowledgements**

This study was a part of a Ph.D. thesis financially supported by Deputy of Research, Iran University of Medical Sciences (Grant No. 93-01-27-24403). Also, the authors declare no conflicts of interest.

**Conflict of Interests**

The authors declare that they have no competing interests.

**References**


8. Kim B, Feldman EL. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Experimental Molecular Med. 2015; 47 (e149): 10.1038/EMM.2015.3


16. Quigley EM. Prebiotics and probiotics; modifying and mining the microbiota. Pharmaco Res. 2010; 61 (213-218): 10.1016/j.phrs.2010.01.004


Probiotics effect on insulin resistance


32. Li X, Song D, Leng SX. Link between type 2 diabetes and Alzheimer’s disease: from epidemiology to mechanism and treatment. Clinical Interven Aaging. 2015;10 (549): dx.doi.org/10.2147/CIA.S74042


http://mjiri.iums.ac.ir
Med J Islam Repub Iran. 2017 (18 Dec); 31:103.