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Paeonol alleviates brain glucose hypometabolism in streptozotocin murine model of sporadic Alzheimer's disease using microPET imaging

Akram Tayanloo-Beik[1], Zahra Kiasalari[2], Mehrdad Roghani[2]*

1. Department of Biology, School of Basic Sciences, Shahed University, Tehran, Iran
2. Neurophysiology Research Center, Shahed University, Tehran, Iran

Abstract

Background and Objective: Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rodents causes a model of sporadic Alzheimer's disease (sAD) with development of insulin resistance and neuronal dysfunction. Paeonol is a phenolic agent with neuroprotective effect due to its anti-inflammatory and anti-oxidant effects. This study was conducted to assess its beneficial effect in prevention of brain glucose hypometabolism in ICV STZ rat model of sAD.

Materials and Methods: STZ (3 mg/kg) was bilaterally injected ICV on days 0 and 2 using stereotaxic surgery and paeonol was orally given at two doses of 25 (low) or 100 (high) mg/kg from day 0 (post-surgery) till day 24 post-STZ. At the end of study, positron emission tomography (PET) imaging was employed with a micro-PET scanner through tail vein injection of 18FDG and brain to background ratio (BBR) was calculated.

Results: ICV-STZ group had a significantly low BBR compared to sham group. In contrast, ICV-STZ group treated with paeonol at a dose of 100 mg/kg had a significantly higher BBR as compared to ICV-STZ group, clearly indicating attenuation of brain glucose hypometabolism. In addition, paeonol at a dose of 25 mg/kg did not significantly improve BBR in ICV-STZ group.

Conclusion: In summary, these results revealed the beneficial dose-dependent effect of paeonol in amelioration of brain glucose hypometabolism in STZ-induced model of sAD.

Keywords: Sporadic Alzheimer's disease, Streptozotocin, Paeonol, Brain glucose hypometabolism, Positron emission tomography

1. Introduction

Alzheimer's disease (AD) is the leading cause of 75% of all dementia cases that commonly appears in the form sporadic or delayed-onset, so-called sporadic Alzheimer's disease (sAD). Neuronal and synaptic loss causes several pathological events like multiple cognitive deficits, oxidative stress, changes in glucose metabolism, and so forth. Accumulated data demonstrated that glucose serves as one of the key players in the pathogenesis of sAD (1-3). Actually, impaired glucose metabolism is known as one of the important risk factors of AD that is characterized by reduced uptake of glucose in the temporal-parietal and posterior cingulate regions. Glucose is the major energy source of the neuronal cells and changes of its metabolism are accompanied by a wide range of pathological conditions (3, 4). One of the well-understood physiological mechanisms is the

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Corresponding Author: Ahmad Asghari **E-mail:** mehjour@yahoo.com

antagonistic regulatory role of insulin on neuronal glucose metabolism. Moreover, since the majority of sAD pathogenic factors like a decrease in the synthesis of neuronal insulin receptors have close similarity with the involved factors of diabetes mellitus, developing animal models that provide more relevant specifications can be beneficial for expanding more researches (3, 5). Among many other models, it has been shown that intracerebroventricular (ICV) injection of streptozotocin (STZ) leads to a valid experimental model of sAD. This valuable model has been widely used for investigating the therapeutic effects of different treatments for AD including drugs (3, 6).

Conventionally, Chinese herbal medicine has been used for thousands of years to treat different diseases. Herein, a phenolic acid compound named paeonol (2′-hydroxy-4′-methoxyacetophenone, C9H10O3) due to its considerable therapeutic impacts like antidiabetic effects has been the core of attention of researchers for diabetes and AD studies. Several studies have shown that paeonol can ameliorate and relieve the energy metabolism in Alzheimer's disease condition (7, 8).

A number of cellular, molecular, and biochemical techniques are commonly used for evaluating the metabolism of glucose in the body. However, emerging more novel diagnostic strategies such as biomarker studies, genetics, and neuroimaging techniques leads to more advances in this field. Incorporating these techniques during development of early signs of AD before its conventional clinical signs is valuable. F-fluorodeoxyglucose positron emission tomography (F-FDG-PET) is a promising neuroimaging tool that measures the cerebral glucose metabolic rate (9-11). Biologically, this is an important indicator of neuronal activity and its alteration is assumed as one of the symptoms of AD. In fact, tracing the modifications that occur in distinct patterns of glucose metabolism is an important manifestation of different stages of AD (9). Therefore, F-FDG-PET can be regarded as a very valuable tool for directly investigating the consequences of different AD treatments in the cerebral cortex (12, 13). With all these in mind, in the present study, the beneficial effect of paeonol treatment in alleviation of brain glucose hypometabolism in STZ-induced rat model of sAD was examined using F-FDG-PET scanning technique.

2. Materials and Methods

2.1. Animals

In this study, 20 male Wistar rats (200-250 g) were purchased from Shahid Beheshti University of Medical Sciences. They had free access to food and water with regulated temperature (21-23°C), light/dark cycle, and humidity (40-50%). This study was approved by Institutional Ethics Committee of the Shahed University.

2.2. Experimental protocol

Animals were divided into 4 groups, each containing 5 rats including; 1- sham group with sham operation and bilateral ICV injection of artificial cerebrospinal fluid (aCSF), 2- ICV STZ group with bilateral injection of STZ, 3- STZ + Paeonol 25 with bilateral ICV injection of STZ and also with paeonol treatment at a dose of 25 mg/kg, and 4. STZ + Paeonol 100 group with bilateral ICV injection of STZ and with paeonol treatment at a dose of 100 mg/kg. Composition of aCSF solution consisted of (in mM): 125 NaCl, 3 KCl, 1.15 CaCl2, 0.8 MgCl2, 27 NaHCO3, and 0.33 NaH2PO4 with pH adjusted to 7.4. Paeonol (SigmaAldrich, USA) was dissolved in 10% Cremophor (SigmaAldrich, USA) and administered up to day 24 after first STZ microinjection, started 2 h after first ICV STZ injection (day 0). STZ was freshly dissolved in aCSF and bilaterally injected using a Hamilton syringe at a dose of 3 mg/kg and, two times with an interval of 48 h (14).

In order to induce sAD model in rats, they were first anesthetized using an i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Then, rats were placed in a stereotaxic apparatus (Stoelting Co., USA) and received ICV injection of STZ (Santa Cruz Biotechnology, Inc., USA) twice at an interval of 48 h. In accordance to Paxinos and Watson stereotaxic atlas, targeting of the lateral ventricles for ICV injection (5 □l/side) was conducted at the following coordinates: - 0.8 mm posterior to bregma, 4 mm below dura, and ± 1.4 mm lateral to bregma. ICV microinjection was done using a Hamilton microsyringe. Following the first STZ injection, treatment with paeonol was initiated 2 h after first STZ injection in a daily manner using a gavage needle till day 24 post-surgery.

2.3. Positron emission tomography (PET) imaging

The small-animal PET scans were obtained with a micro-PET scanner (Xtrim PET). Rats were injected via the tail vein with about 1 mCi of the 18FDG under general anesthesia. For each small animal PET scan, 3 dimensional regions of interest (ROIs) were manually drawn around the brain. The ROI's were converted to brain to background ratio (BBR) as: (ROI counts per voxel) / (background counts per voxel).

2.4. Statistical analysis

All presented data are brought as means ± standard error (SE) and were analyzed by statistical software GraphPad Prism 8.4 (GraphPad Software Inc., USA). Significant differences were found out using one-way analysis of variance with subsequent Tukey's multiple

range test, if required. The level of statistical significance shown in results section was based on the p-values $p<0.05$, $p<0.01$ and $p<0.001$.

3. Results

3.1. The effect of ICV STZ on glucose metabolism using microPET

Brain glucose hypometabolism is regarded as one of the most important pathophysiological attributes of AD in humans and its animal models. To assess brain glucose metabolism in ICV STZ rats, we employed overall 18FDG distribution in the rat brain through microPET and its results were presented as brain to background ratio (BBR) (Figure 1). In this respect, ICV-STZ group had a significantly low BBR when compared with the sham group ($p<0.01$). This clearly indicated a disturbance in brain glucose metabolism following ICV injection of the diabetogenic drug STZ, somehow indicating development of glucose hypometabolism in the brain tissue.

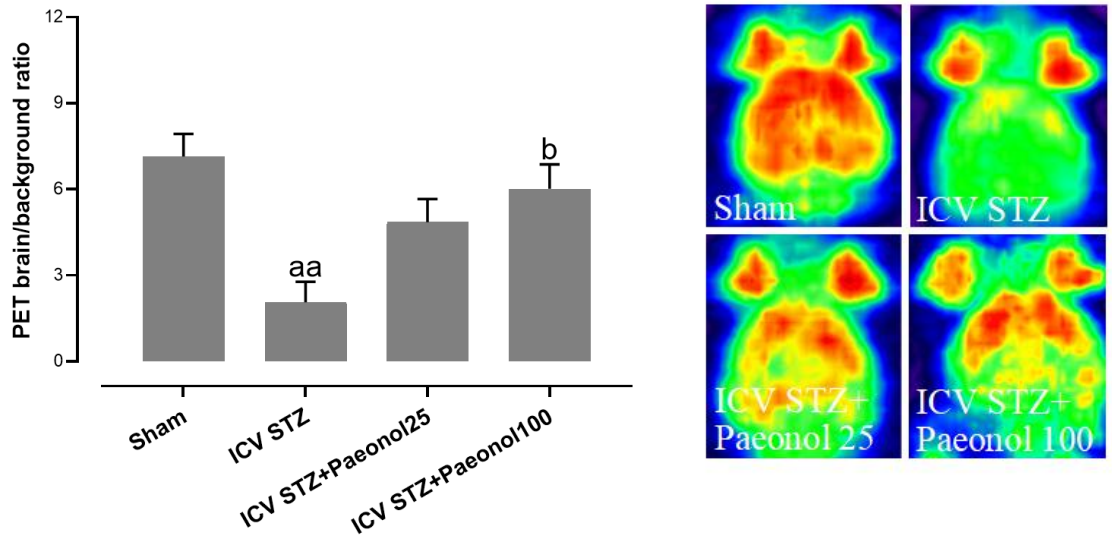

Fig 1. Changes in brain glucose metabolism in microPET images in axial views. Data are expressed as means ± SEM. n = 5 rats/group. [aa] $p < 0.01$versus sham group; [b] $p < 0.05$ versus ICV STZ.

4. Discussion

As one of the novel promising tools that provide early detection of AD with a non-invasive procedure, brain imaging makes it possible to develop more effective therapeutic approaches. Clinically, 18F-FDG-PET is a well-established method for early diagnosis of different types of dementia including AD. Actually, 18F-FDG targets the cerebral glucose metabolism that is one of the definite manifestations of synaptic activity. In other words, it reveals neuronal dysfunction as the previous stage of neuronal loss that leads to early diagnosis of neurodegenerative disorders (13, 15). In present study, using this advantageous tool, we examined brain glucose metabolism pattern in STZ model of sAD in the rat receiving different doses of paeonol.

Paeonol, the main phenolic component of Cortex Moutan, has been shown to possess neuroprotective effects in different models of central nervous system diseases (8, 16-18). Although the most of well-known effects of paeonol are anti-inflammatory and antioxidant activities, there are also evidences for its neuroprotective and hypoglycemic activities in diabetic and neurodegenerative disorder models (16, 19-21). Small molecular weight of paeonol allows it to cross the blood-brain barrier (BBB) and its oral administration cause more rapid distribution to the brain. Moreover, pharmacological studies demonstrated the dose-dependent manner of paeonol uptake but its underlying mechanism is still under investigation (8). According to these pieces of evidence, we used different doses of paeonol via oral administration in our model of sAD. Zhang et al. investigated the therapeutic effects of paeonol-ozagrel conjugate (POC) on ischemic stroke and their study revealed an increase in the number of survival neurons

as well as changes in the levels of oxidative stress and inflammatory markers (22). Zhu et al. studied the antidepressant effects of paeonol and demonstrated its reducing effect on synaptic and dendritic loss and its improvement of behavioral deficits (23). Furthermore, the preventive effect of paeonol on neuroinflammation developed in microglial cells has been reported by Lin et al. (24).

Accordingly, as mentioned previously, since brain glucose metabolism has a direct relation with the number and function of survived neurons, paeonol can be a remarkable candidate for alleviation of impaired glucose metabolism in AD rats. Other studies on rat models of diabetic neuropathy have shown that paeonol treatment resulted in a notable decrease in plasma levels of glucose (16) and a number of investigations proved the significant role of paeonol in improving memory and cognitive deficits that are tightly associated with the survival of hippocampal neurons (18, 25-26). Herein, the results of our study demonstrated that neuronal and synaptic loss in sAD rats is accompanied by altered glucose metabolism in the brain regions. In fact, reduced 18F-FDG uptake within the posterior cingulate cortex occurred in the STZ group after 14 days of induction of sAD model. Several studies revealed the relation between decreased glucose metabolism through the disease progression with behavioral and cognitive decline in AD animal models which provide additional proof on our results (12, 13). Nevertheless, identifying the exact mechanisms underlying the paeonol beneficial effect on the regulation of glucose metabolism in the brain needs more research efforts in the future.

One of the limitations of the present work was the lack of coronal view analysis of microPET images at different planes which is highly recommended to be conducted in future studies.

In conclusion, these results revealed the beneficial effect of paeonol in amelioration of glucose hypometabolism in STZ-induced model of sAD. In addition, this effect of paeonol followed a dose-dependent pattern.

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Conflict of interest

There is no conflict of interest to express.

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