Effects of PPARγ agonist decreasing the IL-1β, IL-6 and TNF-α content in rats on focal cerebral ischemia-reperfusion injury.

Qiuju Li1, Yancang Liu2, Lei Chen2*

1Department of Adult Rehabilitation, Jining No.1 People's Hospital, Jining, Shandong, PR China
2Department of Emergency, Jining No.1 People's Hospital, Jining, Shandong, PR China

Abstract

Objective: To observe the protective effects of PPARγ Pioglitazone agonist on the focal cerebral Ischemia-Reperfusion injury (I/R) and explore its mechanism of action.

Methods: By duplicating the rat Middle Cerebral Artery Block Model of Reperfusion (MCAO/R), respectively by TTC staining method, neural function scoring method to observe the effect of the PPARγ agonist on cerebral infarction volume and behavioral score in rats. And observe the effects of PPARγ agonist on the IL-1β, IL-6 and TNF-α content in rats with brain tissue.

Results: PPARγ agonists are able to reduce the volume and behavior of cerebral infarction in I/R rats and decrease the content of IL-1β (t=7.84, P<0.05), IL-6 (t=5.23, P<0.05) and TNF-α (t=8.41, P<0.05) in brain tissues.

Conclusion: PPARγ agonist has a protective effect on cerebral ischemia reperfusion injury in rats, and its mechanism is related to decreased IL-1β, IL-6 and TNF-α in brain tissues.

Keywords: PPARγ agonist, Ischemia-reperfusion injury, Interlukin 1β, Interlukin 6, Tumor necrosis factor-α.

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Introduction

Peroxisome body growth activated receptor gamma (peroxisome proliferator-activated receptor gamma, PPARγ) belong to the members of the nuclear receptor superfamily, is a kind of ligand activated nuclear transcription factor, which can control a variety of gene transcription and expression, to participate in a variety of physiological and pathological processes in the body. Recent studies have found that PPARγ has a close relationship with ischemic pathological injury, and PPARγ agonist pioglitazone has protective effects on the ischemia reperfusion injury of tissue organs during myocardial, pulmonary and shock [1]. However, the protective effects remain obscure. Therefore, this experiment will use PPARγ agonist pioglitazone in middle cerebral artery ischemia reperfusion (middle cerebral artery occlusion/reperfusion, MCAO/R) injury model of rats treatment to identify the effect of IL-1B, IL-6 and TNF-α content on focal cerebral ischemic Reperfusion injury. The effects of PPARγ agonist on focal cerebral ischemia-reperfusion injury were observed, and its action mechanism was discussed.

Material and Method

Material

Animal: Healthy male Sprague-Daw ley rat, weighing 270–310 g, provided by experimental animal center of Xi an Jiaotong university medical school.

Drug and reagent: PPARγ agonist-pioglitazone was provided by Lianyungang Dyuan Pharmaceutical co., LTD. 2, 3, 5-chlorinated triphenyl tetrazazole (2, 3, 5-triphenyl chloride, TTC) was products of Shanghai chemical reagent. IL-1β, IL-6, TNF-α quantitative ELISA kits were provided by Beijing Huanaratek biomedical technology co., LTD., Coomassie brilliant blue protein quantitative kits, provided by the institute of biological engineering in Nanjing. All other reagents were from domestic analysis.

Main instruments: Bipolar electric condenser (Shanghai medical equipment company). Digital camera (Power Shot A610). Multiskan MK3 enzyme standard (Finnish ThermoLab systems company products), low temperature high-speed centrifuge (Beijing medical centrifuge factory).

Method

Grouping and drug administration: 45 healthy male SD rats were randomly divided into 3 groups, with 12 h fasting and free drinking water before the operation. 1. Five sham
operation groups: surgical exposure alone, separation of the neck, internal and external carotid artery, and non-obstruction.

2. 20 patients with ischemia reperfusion model group, which was prepared by the method of line suppository (2 h) and reconnect (22 h) model, 10 TTC staining and 10 other brain tissue halogenation. 3. Pioglitazone treatment group, 20 was ischemic 2 h and refilled 22 h, 10 TTC staining and 10 were treated with cerebral tissue homogenate. In the first week of treatment group, medicine (pioglitazone tablet 20 mg•kg\(^{-1}\)•d\(^{-1}\), 2 times daily, 1 h before surgery, 1 time after surgery). The control group was filled with the volume of saline.

**Neurological impairment score:** The method of Zea Longa [2] was used to observe and record the symptoms of neurological deficiency before executing rats. The absence of neurologic symptoms was 0 point. The lesion was not fully extended to the lateral forelimb at the end of the tail was 1 point. The rotation sign of the paralyzed side was 2 points. The lesion to the side falls were 3 point. There were four points without spontaneous activity and awareness.

**Determination of biochemical indicators of brain tissue homogenate:** IL-1β, IL-6, TNF-α quantitative by ELISA kit. The content of the corresponding inflammatory factors in tissue halogenation was divided into the content of the inflammatory factors in the tissue protein of each microgram, using Coomassie brilliant blue method mark protein level.

**Statistical treatment**

SPSS 13.0 statistical was used for statistical treatment, and all the experimental data were calculated from the mean or plus or minus standard deviation (\(\bar{x} \pm s\)). The comparison between groups was statistically processed by single factor variance analysis and t-test test.

**Results**

The effects of RPPAR gamma agonist pioglitazone on neurofunctional scores and cerebral infarction volume in rats TTC dyeing result shows, the influence of Sham-operated group by TTC dyeing after brain full red, model group rats nerve function defect symptoms was improved, different level strength obvious enhancement (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Neurological score</th>
<th>Volume of cerebral infarction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated group</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>2.682 ± 0.940</td>
<td>0.319 ± 0.079</td>
</tr>
<tr>
<td>Treatment group</td>
<td>10</td>
<td>1.601 ± 0.811(^*)</td>
<td>0.219 ± 0.081(^*)</td>
</tr>
</tbody>
</table>

Compared with model group, \(P < 0.05\)

**Effects of PPAR\(\gamma\) agonist on the IL-1\(\beta\), IL-6 and TNF-\(\alpha\) content in rats with focal cerebral ischemia-reperfusion injury**

Table 2 showed that pioglitazone can decrease the content of IL-1\(\beta\) (\(t=7.84, P<0.05\)), IL-6 (\(t=5.23, P<0.05\)) and TNF-\(\alpha\) (\(t=8.41, P<0.05\)) in modeling group rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>IL-1(\beta)</th>
<th>IL-6</th>
<th>TNF-(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated group</td>
<td>5</td>
<td>1.693 ± 0.391(^*)</td>
<td>1.061 ± 0.312(^*)</td>
<td>0.460 ± 0.077(^*)</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>2.817 ± 0.681</td>
<td>2.876 ± 0.856</td>
<td>1.105 ± 0.441</td>
</tr>
<tr>
<td>Treatment group</td>
<td>10</td>
<td>1.613 ± 0.332(^*)</td>
<td>1.147 ± 0.280(^*)</td>
<td>0.572 ± 0.207(^*)</td>
</tr>
</tbody>
</table>

Compared with model group, \(P<0.05\)

**Discussion**

Cerebral infarction occurrence and development mechanism are complex, not only result in tissue damage caused by hypoxic-ischemic, but also thrombolysis or the establishment of collateral circulation can cause blood flow recirculation and result in reperfusion damage. The damage is a central part of the organization of peroxide and inflammatory cascade reaction. Therefore, blocking lipid peroxidation and inflammatory response is an important strategy for treating ischemic cerebrovascular diseases. The inflammatory response in ischemic areas after cerebral infarction is mainly manifested in leukocyte aggregation, oxygen free radical generation, lysosomal damage, hydrolytic enzyme formation and the formation of inflammatory mediators. Endothelial cells after cerebral ischemia reperfusion inflammatory response to blood-brain barrier damage and increased brain injury and brain edema, on the basis of this pathophysiological process IL-1\(\beta\), TNF-\(\alpha\), IL-6 represented by a variety of inflammatory cell factors such as medium is out of control, release and formation of the "waterfall effect". IL-1\(\beta\) belongs to IL-1 gene family, mainly exists in the brain, by glial cells, nerve cells and endothelial cell synthesis and secretion, it cannot only activate other cytokines to promote synergy B and T cell and other inflammatory mediators, but also can induce to strengthen adhesion of leukocytes and endothelial cells. TNF-\(\alpha\) is a kind of cytokine with activated macrophages to secrete. And it is a polypeptide hormone, has extensive biological functions, mainly related to inflammatory and immune reaction. TNF-\(\alpha\)secretion in the early cerebral ischemia or synthetic is the main cause of brain infarction [3], it has increased the permeability of vascular endothelial cells and induces cell adhesion factor expression, and so on. TNF-\(\alpha\)-induced leukocyte infiltration after cerebral ischemia, and plays an important role in tissue damage, which can cause a multi-core white blood cell aggregation and activation and release of
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Inflammatory mediators. So by locking the expression of TNF-α can reduce ischemic neuronal damage. IL-1β, TNF-α can also activate other cells to produce multiple cytokines, such as the synthesis of IL-6. IL-6 is a kind of multifunctional single glycoprotein cytokine with a broad range of biological activities, and made up of T cells, fibroblast cells, monocytes, macrophages and endothelial cells. With extensive biological functions, it is mainly participate in the body's immune and inflammatory response, now think IL-6 is a sign of acute ischemic brain damage [4]. This is consistent with the results of this study, which indicates that the application of MCAO model is reliable and verified the above theories.

Pioglitazone is a thiazolidine drug, which is a selective agonist of PPARγ, initially used as an insulin sensitizer for clinical type 2 diabetes. More and more recent studies have shown that these drugs have potential anti-inflammatory and immunomodulatory effects. Now think of these drugs have resistance to ischemic injury and the effect of the activated inhibits the TNF-α, Interferon (IFN)-γ, Intercellular Adhesion Molecule (ICAM)1, IL-6, P-select element (P-select in) such as cytokines, adhesion molecules and reduce its activity.

This study found that pioglitazone can effectively inhibit the expression of IL-1β, TNF-α, IL-6 after cerebral ischemia/reperfusion, thus inhibiting inflammatory reaction after reperfusion, ischemia/reperfusion after brain infarction volume, improve neural function defect scale to demonstrate that pioglitazone on ischemic nerve injury plays a protective role which is similar to other research results reported in the literature in recent years [5,6]. Diabetes is an important independent risk factor for cerebral infarction, so it is of great practical significance to explore the protective effect and the mechanism of such drugs on cerebral infarction.

In conclusion, we found that PPARγ agonist has a protective effect on cerebral ischemia reperfusion injury in rats, and its mechanism is related to decreased IL-1β, IL-6 and TNF-α in brain tissues.

References

*Correspondence to
Lei Chen
Department of Emergency
Jining NO.1 People's Hospital
PR China

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