EFFECTS OF JIANG TANG FANG LONG FORMULA ON INSULIN PRODUCTION AND FUNCTION IN AN ANIMAL MODEL OF DIABETIC HEARING LOSS

Yang YunXia1, LI Ruiyu2, JING Jian Mei2, ZHANG Yanzhuo2, LI Meng3, LI Bin4, FENG Jin Ping2, LI Li2, LI Hui Zhen2

Abstract

Objective To study the influence of Jiang Tang Fang Long formula on insulin production and function in an animal model of diabetic hearing loss. Methods Wistar rats (n=60) were randomly divided into 6 groups (10 in each) to receive no treatment (the normal control, Group A), or to receive intra-peritoneal 55 mg/kg streptozotocin with (Groups C, D and E) or without (Group B) subsequent Jiang Tang Fang Long formula treatment at various doses or Yu Long Wan treatment (Group F). After 60 days, fasting blood glucose (FBG), body weight (BW) and fasting insulin (FINS) were recorded and the HOMA-IR and HOMA-β calculated. Insulin expression in pancreatic tissues was measured by radioimmunoassay. Results Compared with animals that received streptozotocin without rescue treatment (Group B), animals that received higher doses of Jiang Tang Fang Long formula (Groups D and E) showed improved indices of diabetes manifestation (P<0.05) and improved HOMA-β (P<0.05) in a dose-dependent manner, as well as improved insulin expression in pancreatic islets (P<0.05). The difference between low dose Jiang Tang Fang Long formula treatment (Group C) and Group B was not significant (P>0.05). Conclusion Our results suggest that Jiang Tang Fang Long formula may improve pancreatic β-cells function which may explain its efficacy in treating diabetic hearing loss.

Key words: Jiang Tang Fang Long formula, diabetes, deafness, Wistar rats, streptozocin

Introduction

Diabetes can lead to sensorineural deafness. The exact mechanism of diabetic hearing loss is yet to be defined. The authors have used Jiang Tang Fang Long (literally “glucose lowering and deafness preventing”) capsule to treat diabetic patients with hearing loss with some success[1-4]. To study the underlying mechanisms for the efficacy, we designed this study to examine the effects of the formula on insulin production and function in an animal model with streptozotocin (STZ) induced hyperglycemia and hearing loss. The preliminary results are reported.

Materials and methods

The Jiang Tang Fang Long formula

The key ingredients in the Jiang Tang Fang Long formula (designed by the authors) included kudzuvine, red sage root, rhizome of Sichuan lovage, fox glove, Chinese yam, poria cocos, alismatis rhizome, dogwood fruit, magnetite, etc. These were obtained from the Le Ren Tang (an herbal pharmacy) in Shijiazhang with identification as authentic Chinese medicine materials. The formula was prepared in the form of suspension.

Affiliation:
1Department of Laboratory, Xingtai People’ s Hospital, Xingtai 054031,
2Second Affiliated Hospital of Xingtai Medical College, Xingtai 054000
3Medical Team of Hetian Detachment Xinjiang Armed Police, Hetian 848011;
4Department of Biochemistry, Hebei Medical University, Shijiazhuang 050017, China

Corresponding authors:
LI Ruiyu, Email:liruiyu681021@163.com
Animal model
Sixty healthy adult male Wistar rats (200-250g), without noise exposure history were randomly divided into 6 groups (10 in each). One group (Group A) served as the normal control and received no treatment other than equivalent volume of saline. Diabetic deafness was induced in the rest of the animals (Groups B—F) as reported by WANG Shili et al and YU Demin et al[^6]. Briefly, animals were given 55 mg/kg streptozotocin (SIGMA, USA) diluted in 0.1mg/L natrium citricum buffer solution (pH 4.2) by intra-peritoneal injection after fasting for 12 hours. The levels of blood glucose and urine glucose were measured one week later. A diabetic status was established when blood glucose was >14.2mmol/L and urine glucose > (+++). Auditory brainstem response (ABR) thresholds were recorded after two months, and diabetic deafness was confirmed when ABR thresholds were significantly increased.

**ABR tests**

ABR thresholds were recorded on a Traveler Express evoked potential system (Bio-logic, USA). After anesthesia with intra-peritoneal pentobarbital sodium (45 mg/kg), needle electrodes were placed at the middle point between the two pinna (recording), contralateral pinna (reference) and ipsilateral pinna (ground). Stimuli were delivered via a TDH-39P headphone placed 2 cm from external auditory meatus. The bandpass filter was set between 100 and 3000 Hz. Tests were performed in a sound-proof chamber. The wave II threshold, latencies and intervals between ABR waves were measured.

**Rescue treatment with Jiang Tang Fang Long formula and Yu Long Wan**

Animals in Group B received no rescue treatment but equivalent volume of saline. Animals in Groups C, D and E received the Jiang Tang Fang Long formula at 3.3 g/kg/d, 10 g/kg/d or 30 g/kg/d, respectively, and those in Group F received Yu Long Wan at 25 g/kg/d. After 60 days of treatment, the fasting blood glucose (FBG), body weight (BW) and fasting insulin (FINS) levels were measured.

**Determination of index**

A 7080 automated biochemical analyzer (Hitachi, Japan) was used to test FBG. Radioimmunoassay measurement of serum insulin was completed using a test kit from the Nanjing BioTechnolog. The HOMA formula[^7] was used to estimate IR (HOMA-IR) and function of islet beta cells (HOMA-β). For immunohistochemical tests, the animal was sacrificed and pancreatic tissues containing acinar cells were isolated, paraffin-embedded, dehydrated using gradient ethanol and sliced consecutively at 5 μm. After immunohistochemical staining, five sections were randomly selected from each group and 10 randomly selected high power fields under a light microscope were photographed and analyzed using the Image-pro plus Graphics Analysis tool software.

**Statistical Analysis**

The SPSS 11.0 statistical package was used for statistical analysis. The mean and standard error were calculated for measurement data. The Student t test was adopted for comparison between groups. P<0.05 was considered to indicate statistical significance.

**Results**

Compared with no rescue treatment (Group B), Jiang Tang Fang Long formula at higher doses (Groups D and E) improved the diabetic indices in a dose-dependent manner (P<0.05), although the HOMA-β also decreased as the dose increased (P<0.05). The differences between low dose of Jiang Tang Fang Long formula treatment (Group C) and no rescue treatment (Group B) were not statistically significant (P>0.05) (Table 1).

Enhanced insulin expression in islet cells was noticed in Groups D, E and F, as significantly higher average optical density and area percentage of insulin-positive cells (P<0.05), with decreasing average gray as dose in-

<table>
<thead>
<tr>
<th>Group</th>
<th>FBG (mmol/L)</th>
<th>BW (g)</th>
<th>FINS (mmol/L)</th>
<th>(HOMA-IR)</th>
<th>(HOMA-β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.95±1.40**</td>
<td>416.89±22.42**</td>
<td>2.43±0.19**</td>
<td>1.08±0.35**</td>
<td>4.63±0.20**</td>
</tr>
<tr>
<td>B</td>
<td>21.19±3.22</td>
<td>469.03±17.73</td>
<td>3.36±0.18</td>
<td>3.32±0.20</td>
<td>3.50±0.14</td>
</tr>
<tr>
<td>C</td>
<td>19.24±2.60</td>
<td>455.74±25.26</td>
<td>3.22±0.19</td>
<td>2.99±0.25</td>
<td>3.67±0.15</td>
</tr>
<tr>
<td>D</td>
<td>16.69±2.14**</td>
<td>445.18±22.45*</td>
<td>3.10±0.23*</td>
<td>2.69±0.35*</td>
<td>3.73±0.17*</td>
</tr>
<tr>
<td>E</td>
<td>14.78±3.29**</td>
<td>436.60±20.63**</td>
<td>2.99±0.19**</td>
<td>2.48±0.34**</td>
<td>3.84±0.12**</td>
</tr>
<tr>
<td>F</td>
<td>13.47±2.32**</td>
<td>444.74±17.09*</td>
<td>2.85±0.21**</td>
<td>2.25±0.30**</td>
<td>3.95±0.18**</td>
</tr>
</tbody>
</table>

Compared to Group B: * = P<0.05; ** = P<0.01.
Discussions

Sensorineural deafness is common in diabetes. While the underlying mechanisms are not clear, it is obviously important to control the progress of diabetes in order to reduce diabetes related hearing disability. Islet β cells play a key role in insulin production. Insulin and osteocalcin have a close relation. Animal studies have shown that increasing the dose of osteocalcin enhances insulin expression and islet β cells proliferation[8-9]. “Kidney”-tonifying traditional Chinese medicine agents can stimulate the proliferation, differentiation and maturation of osteoblasts. This is believed to correct “kidney deficiency” and related disorders in the “nerve-endocrine-immunity network” and “nerve-endocrine-bone metabolism” [10]. Some evidence has showed that Jiang Tang Fang Long capsule can help adjust osteocalcin levels in patients with diabetes related sensorineural deafness of various traditional Chinese medicine types[11]. The current study aims to study potential mechanisms of this effect in relation to insulin expression in the pancreas and its functions.

In traditional Chinese medicine theories, rhizome of Sichuan lovage, one of the key ingredients in the Jiang Tang Fang Long formula, goes through the twelve regular meridians. Working with Red sage root, it has the action of moving “circulation” to reduce “blood stasis”. Kudzuvine in the formula raises the “lucid yang” and guides medicines. Fox glove softly enriches the “liver” and “spleen dampness”. Alismatis rhizome helps clear “kidney turbidity”.

Dogwood fruit nourishes the “liver” and “kidney”. Heavy magnetite (mainly FeO4) can correct the “kidney-yin deficiency” and “is used by all the people with kidney deficiency, deafness and blurred vision”, as claimed in the Amplification on Canon of Materia Medica. In this context, the Jiang Tang Fang Long formula facilitates movement of “circulation” to reduce “blood stasis”. In addition to improving hearing, it may also improve the whole body functioning in patients with diabetes-related hearing loss and is worth clinical trials.

The test results in this study also suggest that the Jiang Tang Fang Long formula improves insulin production in a dose-dependent manner, probably through improving pancreatic β-cells function, in diabetic deafness. We speculate that this effect is mediated by the increase of osteocalcin secretion. These hypotheses and other issues around the “kidney-bone- diabetes network” theory remain to be verified.

References


(Received Mach,21,2013 )