Original Article
Distribution of pancreatic B cell imaging agent 99mTc-DTPA-NGN2 in the body and animal experimental research on pancreatic B cell functional imaging

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Abstract: Purpose: To explore the feasibility of the application of 99mTc-DTPA-Nateglinide as a nuclear medicine imaging agent for evaluating pancreatic B cell function. Methods: (1) Distribution of the experiment: Forty-two mice were selected and divided into seven groups. Each mice was injected with 3.7 MBq (100 μCi) of 99mTc-DTPA-NGN2 from the vena caudalis and was sacrificed by bloodletting at five minutes, 15 minutes, 30 minutes, one hour, two hours, four hours and six hours, respectively. Then, their tissues and organs such as the heart, liver, spleen, brain, kidneys, bones, small bowels, stomach and pancreas, and blood were collected, weighted, and their radioactivity was tested. Subsequently, the percentage injection dose rate (%ID/g) per gram of tissue was calculated. (2) Imaging experiment: Thirty-five mice were selected and divided into seven groups. Each was injected with 18.5 MBq (100 μCi) of 99mTc-DTPA-NGN2 from the vena caudalis and imaging were conducted at the same time as above. (3) Forty-eight Wistar rats were attained and randomly divided into four groups. The first group served as the healthy control group, while the second, third and fourth groups were diabetic model groups induced by intraperitoneally injecting STZ at different doses. Each group was injected with 99mTc-DTPA-Nateglinide from the vena caudalis, and radiological evaluations were conducted at 30 minutes, one hour, 1.5 hours and two hours, respectively. The data obtained were estimated using a correlation comparison with the levels of insulin and immunohistochemical count of beta cells. Results: The 99mTc-DTPA-Nateglinide demonstrated good imaging in the pancreases of mice and rats, and was positively correlated to the level of insulin and the number of pancreatic beta cells. Conclusion: Pancreatic beta cell imaging using 99mTc-DTPA-Nateglinide may be a method to evaluate pancreatic beta cell function.

Keywords: 99mTc-DTPA-Nateglinide, islet imaging, diabetes mellitus

Introduction
Diabetes is a metabolic disorder caused by an absolute or relative deficiency of insulin. Both type 1 and type 2 diabetes are characterized by deficits of beta cell: around 99% deficit in long-standing type 1 diabetes [1], and 65% deficit in long-standing type 2 diabetes [2]. Type 1 diabetes results from a selective destruction of the beta cells by a T-cell mediated autoimmune process [3]. Type 2 diabetes begins with insulin resistance in the peripheral tissues, a gradual increase in circulating blood glucose that triggers beta cells to release even more insulin, and subsequent failure of beta cells to maintain normal glucose homeostasis [4].

Currently, there is no ideal method for noninvasively measuring changes in functional β-cell function, which would be valuable for assessing diabetes progression, following therapeutic response, or for evaluating the viability of transplanted pancreatic islets [5]. The goal in treating diabetes has turned from decreasing blood glucose to keeping and improving the function of pancreatic beta cells [6, 7]. However, the evaluation of pancreatic islets function is difficult with poor operation, and there are many contradictions and disputes. Noninvasive imaging techniques such as single photon emission computed tomography (SPECT) [8], positron emission tomography [9], bioluminescence imaging [10], and magnetic resonance imaging (MRI) [11-14] have shown promise in detecting BCM. However, these techniques have limitations. For example, β-cell mass can’t reflect function exactly 6. Moore A,

Nateglinide is a drug that clinically promotes the secretion of insulin from pancreatic B cells in patients with diabetes mellitus, and it can be bound to the sulfonylurea receptors of $K_{ATP}$ on the membrane of pancreatic B cells and transported into cells [15], thereby promoting the release of insulin. In theory, nateglinide specifically combines with B cells that have insulin releasing function, making normal functioning B cells to image. However, apoptotic or failed B cells and other normal and pathological tissues of the pancreas could not be imaged; thus, the imaging of $^{99m}$Tc-DTPA-Nateglinide can reflect the function of pancreatic islets.

$^{99m}$Tc-DTPA-NGN2 is one kind of nateglinide that uses DTPA as a chelating agent of labeling matter $^{99m}$Tc. It was used as a nuclear medicine imaging agent for pancreatic imaging to evaluate the function of pancreatic islet beta cells and observe the comparability and correlation between imaging of $^{99m}$Tc-DTPA-Nateglinide and other evaluations of the function of beta cells.

Materials and methods

Materials

The major reagent, DTPA-Nateglinide, was provided by the MD Anderson Cancer Center, University of Texas. The $^{99m}$Mo-$^{99m}$Tc generator was purchased from Beijing Atom Hightech, Ltd. Mice and Wistar rats were purchased from the Experimental Animal Center of The Medical College of Soochow University. The immunohistochemical reagent was purchased from Wuhan Boster biotech, Ltd. Streptozotocin (STZ) was purchased from the SIGMA group. The blood glucose meter was provided by Johnson & Johnson (USA). The rat insulin detection kit was purchased from Shanghai Xitang.

Experimental methods

Labeling of DTPA-NGN2 and determination of the labeling rate: Stannous chloride and freshly rinsed $^{99m}$TcO$_4^-$ was added into the DTPA-NGN2 kit, which was shaken and left standing; thus, labeling was accomplished. Labeling rate was determined by paper chromatography, the expansion agent was a mixed solution of ammonium acetate and methanol at a 4:1 ratio of 1 mol/L. Agents of 95% labeling rate and above can be used in the experiment.

Experiment on the distribution in normal mice body: Forty-two normal mice were attained and divided into seven groups, with six mice in each group. Each was injected with 3.7 MBq (100 μCi) of $^{99m}$Tc-DTPA-NGN2 from the vena caudalis, and was sacrificed by bloodletting at five minutes, 15 minutes, 30 minutes, one hour, two hours, four hours and six hours, respectively; thus, their tissues and organs such as the heart, liver, spleen, brain, kidneys, bones, small bowels, stomach, pancreases and blood were collected, weighted, and their radioactivity was tested. Then, the percentage injection dose rate (%ID/g) per gram of tissue was calculated.

Imaging experiment in normal mice: Five normal mice were injected with 3.7 MBq (100 μCi) of $^{99m}$Tc-DTPA-NGN2 from the vena caudalis, and were sacrificed at five minutes, 15 minutes, 30 minutes, one hour, two hours, four hours and six hours, respectively. Thereafter, tissues and organs were attained and imaging was conducted. The imaging instrument was the coincidence single photon emission computed tomography (SPECT) from PICKER DHC, with a pinhole collimator.

Examination on diabetic rats: Forty-eight eight-week-old normal male Wistar rats were obtained and randomly assigned into four groups, with 12 rats in each group. The first group served as the normal control group, while the second and third or fourth groups served as the diabetic model groups. Fasting blood glucose (fasting for 12 hours) and random blood glucose were detected in all rats. In the present study, random blood glucose was defined as blood glucose in the non-fasting state. A 0.4% STZ solution was prepared using a citric acid-sodium citrate buffer (0.1 mmol/L, pH 4.2) and was used to fast all rats for 12 hours. Thereafter, the second, third and fourth groups of rats were intraperitoneally injected with STZ at body weight doses of 45 mg/kg, 55 mg/kg and 70 mg/kg, respectively. The first group of rats was only injected with the corresponding citric acid buffer. After one week, random blood glucose in rats in the STZ injection group were detected, with the inclusion criteri-
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Table 1. Biological distribution of 99mTc-DTPA-NGN2 in normal mice (%ID/g)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.47±0.19</td>
<td>0.67±0.11</td>
<td>0.39±0.04</td>
<td>0.29±0.05</td>
<td>0.19±0.07</td>
<td>0.19±0.02</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>1.58±0.21</td>
<td>0.89±0.12</td>
<td>0.90±0.35</td>
<td>0.75±0.14</td>
<td>0.51±0.09</td>
<td>0.38±0.08</td>
<td>0.41±0.05</td>
</tr>
<tr>
<td>The spleen</td>
<td>1.18±0.13</td>
<td>0.53±0.13</td>
<td>0.56±0.14</td>
<td>0.42±0.14</td>
<td>0.32±0.04</td>
<td>0.33±0.09</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>2.96±0.50</td>
<td>1.43±0.24</td>
<td>0.69±0.18</td>
<td>0.73±0.05</td>
<td>0.67±0.24</td>
<td>0.45±0.12</td>
<td>0.48±0.23</td>
</tr>
<tr>
<td>Brain</td>
<td>0.21±0.03</td>
<td>0.13±0.03</td>
<td>0.19±0.04</td>
<td>0.17±0.02</td>
<td>0.08±0.03</td>
<td>0.16±0.05</td>
<td>0.12±0.05</td>
</tr>
<tr>
<td>Renal</td>
<td>12.67±4.42</td>
<td>5.18±0.86</td>
<td>3.85±0.49</td>
<td>2.79±0.50</td>
<td>2.01±0.29</td>
<td>1.48±0.27</td>
<td>1.56±0.23</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.04±0.18</td>
<td>0.56±0.09</td>
<td>0.40±0.14</td>
<td>0.28±0.10</td>
<td>0.19±0.08</td>
<td>0.23±0.10</td>
<td>0.15±0.06</td>
</tr>
<tr>
<td>Bone</td>
<td>1.14±0.31</td>
<td>0.70±0.11</td>
<td>0.79±0.19</td>
<td>0.76±0.26</td>
<td>0.73±0.20</td>
<td>0.54±0.21</td>
<td>0.60±0.22</td>
</tr>
<tr>
<td>Sausage</td>
<td>1.45±0.19</td>
<td>0.78±0.24</td>
<td>1.05±0.50</td>
<td>0.93±0.38</td>
<td>0.86±0.29</td>
<td>0.27±0.06</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.61±0.27</td>
<td>0.76±0.09</td>
<td>0.64±0.18</td>
<td>0.61±0.26</td>
<td>0.44±0.20</td>
<td>0.32±0.09</td>
<td>0.34±0.11</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.88±0.22</td>
<td>0.59±0.13</td>
<td>0.86±0.15</td>
<td>1.11±0.09</td>
<td>1.56±0.08</td>
<td>1.02±0.06</td>
<td>0.74±0.08</td>
</tr>
<tr>
<td>Blood</td>
<td>6.17±0.86</td>
<td>2.95±0.23</td>
<td>1.57±0.10</td>
<td>1.00±0.10</td>
<td>0.61±0.13</td>
<td>0.59±0.17</td>
<td>0.55±0.08</td>
</tr>
</tbody>
</table>

The data in the table for: x±s.

Figure 1. Imaging in pancreas became gradually clear over time. In 1-2 hours, pancreatic tissues could be clearly observed.

Results

The distribution of 99mTc-DTPA-NGN2 in normal mice is shown in Table 1. The %ID/g data of tissues and organs revealed that the kidney was the main metabolic pathway of 99mTc-DTPA-NGN2, the brain did not absorb radioactivity, the muscle also absorbed less, the %ID/g of each organ decreased rapidly within one hour after injection, and the %ID/g of the pancreas gradually increased over time; and this was maintained at a high level in 1-2 hours. Imaging results (Figure 1) were consistent with the results of biological distribution experiments, imaging in pancreas became gradually clear with time, and pancreatic tissues could be clearly observed in 1-2 hours.

Histological observation of rat pancreatic islets

In H&E staining under a light microscope, the number of islets was greater in the normal on of a DM model, in which random blood glucose was more than or equal to 11.1 mmol/L continuously for three days; and rats that did not meet this requirements were removed. On the tenth day, 2 ml of blood was collected from the orbit of each rat, and serum was separated to detect the level of insulin. Then, each rat was injected with 3.7 MBq (100 μCi) of 99mTc-DTPA-Nateglinide from the vena caudalis, and was sacrificed after 30 minutes. Rats were dissected, and part of the liver was taken for nuclear medicine determination. The tails of the pancreas were grouped into two: one group was for nuclear medicine determination; and the other group was placed in 10% neutral buffered formalin and fixed in preparation for light microscopic examination and immunohistochemistry. Five immunohistochemical slices were randomly selected, beta cell numbers were counted under high power microscopic view, and their average value was attained. Two diabetic mice were randomly selected and injected with 18.5 MBq (100 μCi) of 99mTc-DTPA-NGN2 from the vena caudalis. Then, they were sacrificed at five minutes, 15 minutes, 30 minutes, one hour, two hours, four hours and six hours, respectively. Thereafter, tissues and organs were collected and imaging was conducted.

Data collation and analysis

All data were analyzed by SPSS 11.0 software, and variables were presented as (x±SD). Variance analysis and t-test were used to compare differences between groups. Correlations between factors were analyzed by Pearson’s related analysis. P<0.05 was considered statistically significant.
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group, the islets were bigger and clear, and the boundaries were distinct. Small and flat cells were surrounding the islets, bigger and round.
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...tissues. The boundaries were not distinct, the diameters of the islets decreased, the number of stained cells was reduced, and vacuoles were presented locally (Figures 4 and 5).

Imaging of 99mTc-DTPA-Nateglinide in rat pancreas was good (Figure 6), and imaging ratio of the pancreas to the liver in different dose groups of rats were significantly different (P<0.05, Tables 2 and 3).

It can be observed that as the dose of STZ increased, β cell count decreased, and damage to pancreatic islet beta cells was aggravated. Following the decrease in insulin level, imaging ratio of the pancreas to the liver diminished. Imaging ratio of the pancreas to the liver detected by 99mTc-DTPA-Nateglinide in different dose groups was positively correlated with insulin level and the number of β cells (Figure 3).

Discussion

In the diagnosis and treatment effect evaluation of diabetes, accurate quantification of beta cells is the key challenge to understanding the loss and dysfunction of beta cells in the course of diabetes [16]. At present, methods for detecting the function of pancreatic islet beta cells are mainly the stimulation of glucose, the insulin secretion stimulated by non-glucose matters, and the secretion function of other substances [17]. Among them, the hyperglycemic clamp technique has been considered as the relevant time in three hours after injection (a total of 23 times) [20]. Compared with the hyperglycemic clamp, it is relatively simple and has good repeatability; but it is also more cumbersome, fever, flushing and other side effects may occur, and blood sampling times may also bring physical pain to patients.

Thus, clinically, the evaluation of the insulin secretion function of beta cells is only a “rough” qualitative analysis. Physicians can only evaluate the function of beta cells in patients with the degree of disease progression, blood glucose and insulin levels, and response to therapy; which has brought lags in diagnosis and treatment [21]. In order to find an objective, simple, noninvasive and inexpensive method to evaluate the function of pancreatic islet cells, 99mTc-DTPA-Nateglinide was used as a nuclear medicine imaging agent to determine the function of pancreatic islet beta cells, and the comparability and correlation between 99mTc-DTPA-Nateglinide imaging and other evaluation methods was observed.

David Yang and Tony Yu, fellows of the MD Anderson Cancer Center, University of Texas, have long been working on the research and development of nuclear medicine imaging agents; and they have successfully developed 99mTc-DTPA-Nateglinide as a radionuclide imaging agent for pancreatic and neuroendocrine tumors; that is, DTPA serves as a bridge between 99mTc and Nateglinide-NGN2, combin-
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Table 2. The fasting blood glucose, radiological imaging of pancreas to liver ratio, serum insulin levels, and beta cell count in each group of rats (X ±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Fasting blood glucose (mmol/L)</th>
<th>Pancreatic and liver ratio</th>
<th>Serum insulin (IU/mL)</th>
<th>β Cell count (β/HP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>5.11±0.45</td>
<td>0.67±0.20</td>
<td>876.0±229.1</td>
<td>64.5±12.73</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>13.55±1.92*,#</td>
<td>0.47±0.25*</td>
<td>448.0±198.3*</td>
<td>40.5±21.83*</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>14.23±2.70*,#</td>
<td>0.37±0.12*,#</td>
<td>320.5±197.43*,#</td>
<td>26.5±11.04*,#</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>14.90±3.90*,#</td>
<td>0.33±0.11*,#</td>
<td>189.0±134.17*,#</td>
<td>24.0±12.94*,#</td>
</tr>
</tbody>
</table>

Note: *compare with 1 P<0.05, #compare with 1 P<0.01.

Table 3. Correlation analysis on data of the radiological imaging of pancreas to liver ratio, serum insulin levels, and beta cell count (r value)

<table>
<thead>
<tr>
<th></th>
<th>Pancreatic and liver ratio</th>
<th>Serum insulin</th>
<th>β cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic and liver ratio</td>
<td>0.716*</td>
<td>0.790*</td>
<td></td>
</tr>
<tr>
<td>Insulin level</td>
<td></td>
<td></td>
<td>0.639*</td>
</tr>
</tbody>
</table>

Note: *P<0.01.

The occurrence of diabetes in rats induced by STZ was dose-dependent [23]. We peritoneally injected different doses of STZ in rats and hoped to observe different degrees of pancreatic beta cell destruction. Results revealed that: 99mTc-DTPA-Nateglinide has good imaging in normal rat pancreas, while imaging has gradually decreased with the increase in damage of pancreatic islet function. At the same time, the level of serum insulin also decreased. The radiological imaging ratio of the pancreas to the liver was positively correlated with the beta cell count. Furthermore, this correlation was more obvious than that between the serum insulin level and beta cell count, which indicate that it has a bright future in clinical application [24].

Diabetes induced by STZ in rats was similar to that of type I. In the future, we would apply this new method to animal models of diabetes type II and explore the correlation between radiological imaging and function of pancreatic islet beta cells in case of insulin resistance.

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