MECHANISM OF FK506-INDUCED GLUCOSE INTOLERANCE IN RATS

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Received November 8, 1993; Accepted February 1, 1994

ABSTRACT — To clarify the mechanism of glucose intolerance induced by FK506, a novel immunosuppressant, 5 or 10 mg/kg/day of FK506 was dosed orally to rats for 2 weeks, and 125I-insulin binding to the erythrocytes, plasma glucose and insulin levels, and pancreatic insulin content were examined. Insulin binding to the erythrocytes of rat dosed with FK506 was similar to that to erythrocytes of the placebo control; Scatchard analysis confirmed that FK506 did not cause damage to the insulin receptor of the erythrocytes. Contrarily, FK506 caused a clear decrease of pancreatic insulin content as well as a slight decrease of plasma insulin level. The results suggest that the glucose intolerance induced by FK506 is associated with a decrease of insulin secretion, but is not associated with impairment of the insulin receptor.

KEY WORDS: FK506, Glucose-intolerance, Insulin receptor, Rat erythrocytes.

INTRODUCTION

FK506 is a neutral macrolide consisting of 23 aliphatic heterocyclic rings. The drug is a potent immunosuppressant which inhibits the production of interleukin (IL)-2, IL-3, and interferon (IFN) in the CD4+ T (helper) cells (Kino et al., 1987; Yoshimura et al., 1989) in the same way as Ciclosporin-A (CsA).

Pharmacologically, FK506 showed excellent effects on such allograft rejections as skin, heart and kidney in various animals (Ochiai et al., 1987a and 1987b; Inamura et al., 1988; Todo et al., 1988), and clinically the drug was effective for the treatment of allograft rejection of the liver, kidney, and pancreas (Starzl et al., 1989).

Various side effects of FK506 have been observed, however, in animal studies (Ohara et al., 1990) and in clinical trials (Armitage et al., 1992). One of them is pancreatic toxicity. We have demonstrated glucose intolerance with low serum insulin levels and low pancreatic insulin content in rats dosed orally with FK506 for 14 days, and vacuoles in β cell of Langerhans islets in these rats (Hirano et al., 1992). Electron microscopy showed that these vacuolations were due to dilation of the endoplasmic reticulum (not shown data). Therefore, we suggest that the glucose intolerance induced by FK506 would be caused through impairment of insulin secretion and suppression of insulin synthesis in the pancreas. Apart from the secretin and synthesis of insulin, the insulin receptor has other important roles in the regulation of blood glucose levels. However, it is not known whether FK506 affects the insulin receptor of rat. In an attempt to elucidate the mechanisms of FK506-induced glucose intolerance, we examined its insulin binding to the erythrocytes of rat, and their plasma and pancreatic insulin levels.

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MATERIALS AND METHODS

1. Experimental design.

Male Jcl : Sprague-Dawley rats (CLEA Japan, Inc., Shiga, Japan) aged 7 weeks and weighing 252–319 g were used in groups of 7 after acclimation to laboratory conditions for one week and more. For dosing, the solid dispersion formulation (Fujisawa Pharmaceutical, Osaka, Japan) of FK506 (Fig. 1) was suspended in distilled water. 5 or 10 mg/kg of FK506 was given orally to rats in a volume of 5 ml/kg once a day for 14 days.

![Chemical structure of FK506.](image)

As the inactive ingredient, placebo of FK506 solid dispersion formulation was given in the same way as the active substance. After the completion of dosing, blood samples were taken from the abdominal artery of each animal to determine plasma glucose, plasma immunoreactive insulin and insulin receptors of the erythrocytes, and the pancreas was isolated to measure pancreatic insulin content.

2. Plasma glucose and insulin assay.

Plasma glucose was measured by the glucose oxidase method using glucose B test kit (Wako Pure Chemicals, Osaka, Japan), and plasma insulin was measured by enzyme immunoassay using Grazyme Insulin EIA test kit (Wako Pure Chemicals, Osaka, Japan).

3. Extraction of insulin from pancreas.

Pancreatic insulin was extracted by homogenizing the pancreas with ethylalcohol containing 0.7N HCl (Helmchen et al., 1984) and centrifuged. Insulin content in each supernatant was measured by the method of EIA as described above.

4. Assay of insulin binding to erythrocytes.

Insulin binding to the erythrocytes was measured according to the method of Gambhir (Gambhir et al., 1978).

Erythrocytes were separated by centrifuging the blood specimens, and suspended in buffer-G (50 mM HEPES, 50 mM Tris, 50 mM NaCl, 10 mM MgSO$_4$, 5 mM KCl, 10 mM CaCl$_2$, 2 mM EDTA, 10 mM Glucose, 1% BSA at pH 8.0) to make $4 \times 10^9$ cells/ml.

50 μl $^{125}$I-insulin (3 ng/ml as insulin with a specific activity of 13.7 MBq $^{125}$I/g of insulin : Daiichi Chemicals, Tokyo, Japan) was added to each 400 μl of erythrocyte suspension, and each mixture was incubated at 15°C for 210 minutes. The incubation was stopped by removing 200 μl portions from each mixture into a microfuge tube to which 500 μl of buffer-G and 200 μl of dibutylphthalate was added beforehand.

The erythrocytes were separated by centrifuging the microfuge tubes. Since the specific gravity of dibutylphthalate is intermediate between the buffer and cells, three layers arise after centrifugation: a buffer layer on the top, an oily layer in the middle, and the cell pellets on the bottom. The cells were then removed and their radioactivity was measured (A). Total radioactivity (B) was also measured for each reaction mixture including the erythrocytes to calculate percent of insulin binding to the erythrocytes according to the following formula:

$$\text{Percent of insulin binding} = \frac{A}{B} \times 100$$

In addition, Scatchard analysis was performed by using binding percent of insulin to the erythrocytes.

RESULTS

1. Plasma glucose and insulin levels, and pancreatic insulin contents.

Plasma glucose levels were higher with significant difference in the rats dosed with 10 mg/kg FK506 as compared with the placebo group, whereas plasma insulin was dose-dependently lower, but without significant difference from the
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Table 1. Plasma glucose and insulin levels, and pancreatic insulin content in rats treated with FK506.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma insulin (μU/ml)</th>
<th>Pancreatic insulin (μU/mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>177 ± 8.3</td>
<td>74 ± 23.8</td>
<td>3857 ± 240</td>
</tr>
<tr>
<td>FK506 (5)</td>
<td>162 ± 12.7</td>
<td>63 ± 17.4</td>
<td>1520 ± 166**</td>
</tr>
<tr>
<td>FK506 (10)</td>
<td>220 ± 13.7*</td>
<td>20 ± 4.9</td>
<td>1441 ± 71.8**</td>
</tr>
</tbody>
</table>

Significance of difference between the placebo control and treatment groups were evaluated by Student's t-test.

*P < 0.05, **P < 0.01.

Placebo group. Correspondingly, pancreatic insulin also decreased: contents were 39% and 30% respectively of those of the placebo group in rats dosed with 5 and 10 mg/kg of FK506 (Table 1).

2. Insulin receptor on erythrocytes.

The effect of FK506 on insulin receptor was examined in vitro using the erythrocytes obtained from rats dosed with 5 or 10 mg/kg/day of FK506. Figure 2 shows the displacement plots of the bound $^{125}$I-insulin on non-labeled insulin binding to the erythrocytes of rat dosed with FK506. The percentages of bound $^{125}$I-insulin at low concentrations of non-labeled insulin were slightly higher for the FK506 5 mg/kg group, but were not dose-dependent, and the displacement curves converged at higher concentration. Using the data from displacement assay, Scatchard analysis was performed to examine the affinity to and the number of insulin receptors. Although it is difficult to calculate the exact binding affinity from curvilinear Scatchard plots (Kahn et al., 1974; Meyts et al., 1973), the plots of the FK506 treated groups coincided with those of the placebo group and the steep slope of curves obtained from the FK506 treated rats indicated that the overall binding affinity would be almost the same as that in the placebo control group. Furthermore, as the terminal slopes of these plots represent the total number of receptor sites present, the results of Scatchard analysis demonstrate that the erythrocytes of all three groups would have approximately the same number of receptor sites per cell.

DISCUSSION

We have already reported that 5 and 10 mg/kg of FK506 caused glucose intolerance associated with decrease of insulin secretion from the pancreas and pancreatic insulin content in rats (Hirano et al., 1992). These results suggest that glucose intolerance induced by FK506 would occur through injury of β cells in Langerhans islets such as vacuolation. Like FK506, CsA has also induced pancreatic toxicity with glucose intolerance, which was associated with a decrease of pancreatic insulin content and vacuolation in β cells (Hahn et al., 1986; Helmchen et al.,...
1984). Additionally, CsA induced a decrease in the affinity of insulin binding in the erythrocytes of rats dosed i.p. with 30 mg/kg/day for one week (Nakagawa et al., 1990). It was concluded that glucose intolerance by CsA occurred through an alteration of the insulin receptor as well as injuries to β cells. To date, the effects of FK506 on insulin receptor(s) have not been investigated. Generally, adipocytes, muscle cells or monocytes are used to examine insulin receptor. In the present study, however, we used erythrocytes for assaying insulin receptors because of the similarity of the receptor between erythrocytes and such nucleated cells as adipocytes, muscle cells and monocytes (Kobayashi et al., 1980). When our binding data were subjected to Scatchard analysis, curvilinear plots were obtained, accordingly, plural insulin receptors were considered to exist on the erythrocytes as well as the adipocytes of rat as suggested by Olesfky (1976), and on the monocytes of human (Bar et al., 1976). 5 and 10 mg/kg of FK506, which induced a clear glucose intolerance in our previous study (Hirano et al., 1992), did not affect either affinity or number of high or low affinity insulin receptors. Plasma insulin levels and pancreatic insulin content decreased in the present study after FK506 administration as we previously demonstrated.

Although the mechanism of “suppression of insulin secretion” and “decrease of pancreatic insulin content” induced by FK506 is unclear, the results of the present study suggest that glucose intolerance induced by FK506 would be due to a disfunction of insulin secretion and synthesis, but not to injury of insulin receptor.

As shown in present study, 5 and 10 mg/kg/day of FK506 induced a pancreas toxicity in rats. These dosages are very much higher than the clinical dose, which is 0.15 mg/kg/day. But hyperglycemia have occurred even at low dose in some of patient (Fung et al., 1991). This suggests FK506 has pancreatic endocrine toxicity in human as well as that in rats. Accordingly patients should be monitored for blood glucose level during treatment of the drug.

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