Combination effects of pioglitazone and bofutsushosan on body weight and blood glucose levels in diabetic KKA\textsuperscript{Y} mice

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Abstract

It is well known that obesity is a crucial risk factor to cause insulin resistance and to develop type 2 diabetes. Recently, thiazolidinediones (TZDs), which promote adipocyte differentiation and decrease blood glucose levels by improving insulin sensitivity, have been developed for the treatment of type 2 diabetes. However, body weight gain has often been observed, especially in effective cases, after long-term treatment with TZDs, raising the problem that the hypoglycemic effects of TZDs might be reduced by such body weight gains. In the present study, the effects of pioglitazone (PIO), a representative TZD, on body weight and blood glucose levels in KKA\textsuperscript{Y} mice, an animal model of type 2 diabetes, were investigated when PIO was administered for 4 weeks either alone or in combination with bofutsushosan (BOF), a Kampo medicine used to treat obesity. Although the body weight of the group treated with PIO alone was already significantly higher than that of the control group after 1 week of treatment, the serum glucose level of the PIO-treated group was still significantly lower than that of the control group at this time point. However, the body weight increased further and the hypoglycemic effect was lost in the group treated with PIO alone after 4 weeks of treatment. On the other hand, in the group treated with PIO in combination with BOF, such body weight gain was inhibited and the hypoglycemic effect was maintained throughout the 4-week treatment period. These results suggest that the hypoglycemic effect of PIO can be maintained for a long period without body weight gain if PIO is used in combination with BOF.

Key words  pioglitazone, bofutsushosan, diabetic mice, body weight, blood glucose.
Abbreviations  BOF, bofutsushosan; GPT, glutamate-pyruvate transaminase; PIO, pioglitazone; TG, triglyceride; TZD, thiazolidinedione.

Introduction

Type 2 diabetes is generally characterized by insulin resistance in skeletal muscles or adipose tissues. Such insulin resistance is caused mainly by obesity arising from overeating and/or insufficient physical exercise as well as a genetic predisposition. Recently, various thiazolidinediones (TZDs) have been developed for the treatment of type 2 diabetes. TZDs are known to promote adipocyte differentiation through the activation of nuclear peroxisome proliferator-activated receptor (PPAR)\textsubscript{\gamma} and to decrease blood glucose levels by improving insulin sensitivity.\textsuperscript{1)} Although TZDs are remarkably effective against hyperglycemia, they often cause body weight gain with long-term treatment, especially in effective cases. Such body weight gain resulting from TZD administration is presumed to reduce the hypoglycemic effects of TZDs.\textsuperscript{2,3)} Since obesity is an essential cause of insulin resistance in type 2 diabetes.
To approach this problem, several drugs expected to prevent TZD-induced body weight gain were used in combination with TZDs. For example, the α-glucosidase inhibitor voglibose, the biguanide drug metformin and the hypolipidemic drug fenofibrate were reported to inhibit TZD-induced body weight gain in diabetic animals. However, the treatment periods during which these drugs were administered in combination with TZDs were only about 2 weeks. Therefore, whether these drugs are capable of inhibiting TZD-induced body weight gain and maintaining the hypoglycemic effects of TZDs when administered for extended periods remains unclear. Moreover, metformin itself is associated with the risk of several adverse effects including diarrhea and lactic acidosis, although its administration reportedly prevents TZD-induced body weight gain in clinical cases.

Bofutsushosan (BOF) is a Kampo medicine used to treat obesity, and its anti-obese and anti-diabetic effects have been confirmed in various animal models. In the present study, the effects of pioglitazone (PIO), a representative TZD, on body weight and blood glucose levels in KKA mice, an animal model of type 2 diabetes used in our previous study, were investigated when PIO was administered for 4 weeks either alone or in combination with BOF.

**Materials and Methods**

**Animals:** Male KKA/Ta mice and male C57BL/6J mice were used as obese diabetic animals and normal animals (for reference), respectively. The mice were 5 weeks old at the time of their purchase from Clea Japan (Tokyo, Japan); the animals were used in the experiments after 2 weeks of acclimation and were housed individually in an air-conditioned room (23 ± 2°C, humidity: 55 ± 10%) under a 12-hour dark/12-hour light cycle (lights on at 8:00 - 20:00) and were given free access to water and food. This study was approved by the Animal Experiment Committee of Kampo Research Laboratories, Kracie Pharma, Ltd., in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

**Drugs:** Actos Tablets (Lot No. 0004; Takeda Pharmaceuticals Co., Ltd., Osaka, Japan), which was purchased from Kuraya Sanseido Inc. (Tokyo, Japan) for this study, was used as PIO. The tablets were ground into powder before use. The BOF (Lot No. 93171-0; Kracie Pharma, Ltd., Tokyo, Japan) was a dried extract powder obtained from a mixture of crude drugs as follows: Angelicae Radix, Paeoniae Radix, Cnidii Rhizoma, Gardeniae Fructus, Forsythiae Fructus, Menthae Herba, Schizonepetae Spica, Saposnikoviae Radix and Ephedrae Herba (weight ratio of 1.2 each); Atractylodis Rhizoma, Platycodi Radix, Scutellariae Radix, Glycyrrhizae Radix and Gypsum Fibrosum (weight ratio of 2.0 each); Talcum (weight ratio of 3.0); Zingiberis Rhizoma (weight ratio of 0.4); Rhei Rhizoma (weight ratio of 1.5); and dried sodium sulfate (weight ratio of 0.75) instead of mirabilite. The three-dimensional HPLC profile of BOF is shown in Fig. 1.

![Fig. 1 Three-dimensional HPLC profile of bofutsushosan](image-url)
Experimental procedures: Powdered diets (CE-2; Clea Japan, Tokyo, Japan) containing PIO and/or BOF were freely given to KKA\(^y\) mice for 4 weeks. The ratio of each drug in the powdered diets was adjusted every other day based on body weight and food intake to obtain a dose of 5 mg/kg/day for PIO and 4.3 or 6.4 g/kg/day for BOF; the averages of the drug ratios were 0.004% for PIO and 3.4% or 5.0% for BOF, respectively. Plain CE-2 was freely given to control KKA\(^y\) mice and normal C57BL mice for 4 weeks. The serum parameters were measured once a week after collecting blood from the orbital plexus under ether anesthesia. After the final blood collection, the mice were housed individually in metabolic cages (KN-645; Natsume Seisakusho Co., Ltd., Tokyo, Japan) and water intake and urine volume was measured for 24 hours while the mice had free access to food and water. Subsequently, the mice were sacrificed by exsanguination under ether anesthesia, and the adipose tissues (epididymal, retroperitoneal and inguinal) and liver were removed and weighed. The liver tissues were stored at \(-40^\circ\text{C}\) until further analysis.

Measurement of serum parameters: The serum glucose and glutamate-pyruvate transaminase (GPT) levels were measured using the Glucose B-Test Wako and STA-Test Wako (Wako Pure Chemicals, Osaka, Japan), respectively. The serum insulin level was measured using the Insulin ELISA-kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Measurement of liver triglyceride content: The total lipids in the liver were extracted using the method of Folch et al.\(^{18}\) and were then solubilized with 2-propanol containing 10% Triton X-100. Subsequently, the triglyceride (TG) concentrations in these samples were measured using the Triglyceride E-Test Wako (Wako Pure Chemicals), and the liver TG contents were calculated using the resulting values.

Statistical analysis: The results were expressed as the mean ± S.E.M. The statistical difference between each group (except the normal group) was assessed using the Tukey-Kramer test using StatView Version 5.0 software (SAS Institute Japan Ltd., Tokyo, Japan) and was judged significant when the \(p\) value was less than 0.05 (\(p<0.05\)).

Results

Effects on body weight: Body weight and the body weight gain in each group after 1 week and 4 weeks of treatment are shown in Table 1. Body weight and the body weight gain of the group treated with PIO (5 mg/kg/day) alone (i.e., PIO group) were significantly higher than those of the control group at both time points. However, both the body weight and the body weight gain of the groups treated with PIO in combination with 4.3 or 6.4 g/kg/day of BOF (i.e., PIO+BOF4.3 group or PIO+BOF6.4 group, respectively) were lower than those of the PIO group, with a significant difference noted in the PIO+BOF6.4 group, in a manner that was dependent on the BOF dose at each time point. No differences in body weight or body weight gain were observed between the group treated with BOF (6.4 g/kg/day)

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 week</td>
<td>1 week</td>
</tr>
<tr>
<td>KKA(^y)</td>
<td>Control</td>
<td>7</td>
<td>35.7 ± 0.4</td>
<td>38.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>PIO alone</td>
<td>7</td>
<td>35.9 ± 0.3</td>
<td>41.1 ± 0.3**</td>
</tr>
<tr>
<td></td>
<td>PIO + BOF 4.3</td>
<td>7</td>
<td>35.9 ± 0.1</td>
<td>39.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>PIO + BOF 6.4</td>
<td>7</td>
<td>35.4 ± 0.5</td>
<td>36.9 ± 0.3**</td>
</tr>
<tr>
<td></td>
<td>BOF 6.4 alone</td>
<td>7</td>
<td>35.6 ± 0.4</td>
<td>36.9 ± 0.5**</td>
</tr>
<tr>
<td>C57BL</td>
<td>Normal</td>
<td>7</td>
<td>24.9 ± 0.3</td>
<td>25.3 ± 0.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. PIO: pioglitazone 5 mg/kg/day; BOF 4.3: bofutsusosan 4.3 g/kg/day; BOF 6.4: bofutsusosan 6.4 g/kg/day. The statistical difference between each group (except the normal group) was assessed using the Tukey-Kramer test: *\(p<0.05\), **\(p<0.01\), compared with the control group; \(\dagger p<0.05\), \(\ddagger p<0.01\), compared with the PIO alone group.
alone (i.e., BOF6.4 group) and the control group at either
time point.

**Effects on food intake:** The cumulative food intakes of
each group after 1 week and 4 weeks of treatment are
shown in Table 2. No differences in cumulative food in-
take were observed between the PIO group and the con-
trol group at either time point. However, those of the
PIO+BOF4.3 and PIO+BOF6.4 groups were lower than
that of the PIO group, with a significant difference
noted in the PIO+BOF6.4 group, in a manner that was
dependent on the BOF dose at both time points. The cu-
mulative food intakes of the PIO+BOF6.4 and BOF6.4
groups were significantly lower than that of the control
group after 4 weeks of treatment.

**Effects on serum glucose:** The serum glucose levels of
each group after 1 week and 4 weeks of treatment are
shown in Fig. 2. After 1 week of treatment, the serum
glucose level of the PIO group was significantly lower
than that of the control group. The serum glucose levels
of the PIO+BOF4.3 and PIO+BOF6.4 groups were
lower than that of the PIO group, with a significant dif-
ference noted in the PIO+BOF6.4 group, in a manner
that was dependent on the BOF dose.

On the other hand, no differences in the serum glu-
cose level were observed between the PIO group and the
control group after 4 weeks of treatment. However,
similar to the situation after 1 week, the serum glucose
levels of the PIO+BOF4.3 and PIO+BOF6.4 groups
were lower than that of the PIO group in a manner that
was dependent on the BOF dose. In particular, the
serum glucose level of the PIO+BOF6.4 group was sig-
ificantly lower than not only that of the PIO group, but
also that of the control group.

The serum glucose level of the BOF6.4 group was
significantly lower than that of the control group after 1
week and 4 weeks of treatment, although it was slightly
higher than that of the PIO+BOF6.4 group at both time
points.

**Effects on serum insulin:** The serum insulin levels of
each group after 1 week and 4 weeks of treatment are
shown in Fig. 3. After 1 week of treatment, the serum
insulin level of the PIO group was lower than that of the
control group (decreased by 37% of the control group),
but the difference was not significant. The serum insulin
levels were also lower in the PIO+BOF4.3 and
PIO+BOF6.4 groups than in the control group, with a
significant difference noted in the PIO+BOF6.4 group.

However, such decreases were not dependent on the
BOF dose.

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**Table 2** Combination effects of pioglitazone and bofutsushosan on food intake in KKA\(^y\) mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>N</th>
<th>Cumulative food intake (g/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0–1 week</td>
</tr>
<tr>
<td>KKA(^y) Control</td>
<td>7</td>
<td>47.4 ± 1.3</td>
<td>159.7 ± 4.0</td>
</tr>
<tr>
<td>PIO alone</td>
<td>7</td>
<td>52.0 ± 0.7</td>
<td>163.1 ± 3.7</td>
</tr>
<tr>
<td>PIO + BOF 4.3</td>
<td>7</td>
<td>45.6 ± 1.7</td>
<td>146.4 ± 4.9</td>
</tr>
<tr>
<td>PIO + BOF 6.4</td>
<td>7</td>
<td>41.3 ± 0.7(#)</td>
<td>134.9 ± 2.5(#)</td>
</tr>
<tr>
<td>BOF 6.4 alone</td>
<td>7</td>
<td>40.7 ± 3.4(#)</td>
<td>135.9 ± 5.6(#)</td>
</tr>
<tr>
<td>C57BL Normal</td>
<td>7</td>
<td>32.7 ± 1.5</td>
<td>106.9 ± 4.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. PIO: pioglitazone 5 mg/kg/day; BOF 4.3: bofutsushosan 4.3 g/kg/day; BOF 6.4:
bofutsushosan 6.4 g/kg/day. The statistical difference between
each group (except the normal group) was assessed using the
Tukey-Kramer test: \(\#\) \(p<0.01\), compared with the control group;
\(\#\) \(p<0.01\), compared with the PIO alone group.

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**Fig. 2** Combination effects of pioglitazone and
bofutsushosan on serum glucose levels in KKA\(^y\)
mice

Each column and bar represents the mean ±
S.E.M., \(n=7\). P: pioglitazone 5 mg/kg/day; B:
bofutsushosan 4.3 or 6.4 g/kg/day; N: normal
mice (for reference). The statistical difference
between each group (except the normal group)
was assessed using the Tukey-Kramer test:
\(\#\) \(p<0.01\), compared with the control group
(P: --; B: --); \(\#\) \(p<0.05\), \(\#\) \(p<0.01\), compared with
the PIO alone group (P: +; B: -).
On the other hand, no differences in the serum insulin level were observed between the PIO group and the control group after 4 weeks of treatment (decreased by 13% of the control group). Similar results were observed in the PIO+BOF4.3 and PIO+BOF6.4 groups.

No differences in the serum insulin level were observed between the BOF6.4 group and the control group at either time point.

**Effects on adipose tissue weight:** The adipose tissue weights of each group after 4 weeks of treatment are shown in Table 3. Regarding the amount of visceral fat, no differences in retroperitoneal adipose tissue weight were observed among the groups. However, the epididymal adipose tissue weight of the PIO group was slightly lower than that of the control group (decreased by 8% of the control group). The epididymal adipose tissue weights of the PIO+BOF4.3 and PIO+BOF6.4 groups were also slightly lower than that of the control group but were almost equal to that of the PIO group.

On the other hand, the weight of the inguinal adipose tissue, a subcutaneous fat, of the PIO group tended to be higher than that of the control group (increased by 21% of the control group). However, the inguinal adipose tissue weights of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than that of the PIO group in a manner that was dependent on the BOF dose.

No differences in the weight of each adipose tissue were observed between the BOF6.4 group and the control group.

**Effects on hepatic parameters:** The liver weight, TG content and serum GPT levels of each group after 4 weeks of treatment are shown in Fig. 4. The liver weight of the PIO group was significantly higher than that of the control group. However, the liver weights of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than that of the PIO group in a manner that was dependent on the BOF dose. In particular, the liver weight of the PIO+BOF6.4 group was significantly lower than that of

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**Table 3** Combination effects of pioglitazone and bofutsushosan on adipose tissue weight in KKA\(^\text{a}\) mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>N</th>
<th>Visceral fat (g)</th>
<th>Subcutaneous fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epididymal</td>
<td>Retroperitoneal</td>
</tr>
<tr>
<td>KKA(^\text{a})</td>
<td>Control</td>
<td>7</td>
<td>1.64 ± 0.07</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>PIO</td>
<td>7</td>
<td>1.51 ± 0.04</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>PIO + BOF 4.3</td>
<td>7</td>
<td>1.45 ± 0.06</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>PIO + BOF 6.4</td>
<td>7</td>
<td>1.43 ± 0.05</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>BOF 6.4</td>
<td>7</td>
<td>1.54 ± 0.10</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>C57BL</td>
<td>Normal</td>
<td>7</td>
<td>0.29 ± 0.03</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. PIO: pioglitazone 5 mg/kg/day; BOF 4.3: bofutsushosan 4.3 g/kg/day; BOF 6.4: bofutsushosan 6.4 g/kg/day. The statistical difference between each group (except the normal group) was assessed using the Tukey-Kramer test: no differences were observed among the groups.
the PIO group and was almost equal to that of the control group. The liver weight of the BOF6.4 group was almost equal to that of the control group.

The liver TG content of the PIO group was markedly higher than that of the control group. However, the liver TG contents of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than that of the PIO group in a manner that was dependent on the BOF dose. In particular, the liver TG content of the PIO+BOF6.4 group was significantly lower than that of the PIO group. No differences in the liver TG content were observed between the BOF6.4 group and the control group.

The serum GPT level of the PIO group tended to be higher than that of the control group (increased by 19% of the control group). However, the serum GPT levels of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than that of the PIO group in a manner that was dependent on the BOF dose. In particular, the serum GPT level of the PIO+BOF6.4 group was significantly lower than that of the PIO group and tended to be lower than that of the control group. The serum GPT level of the BOF6.4 group was almost equal to that of the PIO+BOF6.4 group.

**Effects on water intake and urine volume:** The water intake and urine volume of each group after 4 weeks of treatment are shown in Table 4. No significant differences in water intake were observed between the PIO group and the control group. However, the water intake of the PIO+BOF6.4 group was significantly lower than that of the control group (decreased by 37% of the control group). The water intake of the BOF6.4 group tended to be lower than that of the control group (decreased by 20% of the control group) but was higher than that of the PIO+BOF6.4 group.

The urine volume of the PIO group was higher than that of the control group (increased by 30% of the control group), but the difference was not significant. The urine volume of the PIO+BOF6.4 group was lower than that of the PIO group (decreased by 56% of the PIO group), but this difference also was not significant. The urine volume of the PIO+BOF6.4 group was lower than that of the control group (decreased by 43% of the control group). The urine volume of the BOF6.4 group also tended to be lower than that of the control group (decreased by 32% of the control group).

**Discussion**

In the present study, although the body weight of the PIO group was already significantly higher than that of the control group after 1 week of treatment, the serum glucose level of the PIO group was still significantly lower than that of the control group at this time point. However, the body weight was further increased and the hypoglycemic effect was lost in the PIO group after 4 weeks of treatment. On the other hand, such body

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**Table 4** Combination effects of pioglitazone and bofutsushosan on water intake and urine volume in KKAα mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>N</th>
<th>Water intake (mL/24 hours)</th>
<th>Urine volume (mL/24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKAα</td>
<td>Control</td>
<td>7</td>
<td>11.6 ± 0.9</td>
<td>5.97 ± 1.56</td>
</tr>
<tr>
<td></td>
<td>PIO alone</td>
<td>7</td>
<td>10.3 ± 1.4</td>
<td>7.74 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>PIO + BOF 6.4</td>
<td>7</td>
<td>7.3 ± 1.1</td>
<td>3.39 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>BOF 6.4 alone</td>
<td>7</td>
<td>9.3 ± 0.5</td>
<td>4.03 ± 0.46</td>
</tr>
<tr>
<td>C57BL</td>
<td>Normal</td>
<td>7</td>
<td>3.6 ± 0.2</td>
<td>0.14 ± 0.06</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. PIO: pioglitazone 5 mg/kg/day; BOF 6.4: bofutsushosan 6.4 g/kg/day. The statistical difference between each group (except the normal group) was assessed using the Tukey-Kramer test: *p<0.05, **p<0.01, compared with the control group.
weight gain was inhibited and the hypoglycemic effect was maintained in the PIO+BOF4.3 and PIO+BOF6.4 groups throughout the 4-week treatment period in a manner that was dependent on the BOF dose. No significant differences in food intake were observed between the PIO group and the control group throughout the experimental period. However, the food intakes of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than that of the PIO group in a manner that was dependent on the BOF dose. The changes in food intake in the PIO+BOF4.3 and PIO+BOF6.4 groups were consistent with those in the body weight and serum glucose levels in these groups. Moreover, the food intake of the BOF6.4 group was lower than that of the control group throughout the 4-week treatment period. These results suggest that the hypoglycemic effect of PIO is maintained as a result of the inhibition of body weight gain in the combined PIO and BOF treatment groups in response to a decrease in food intake induced by BOF treatment. On the other hand, BOF is known to enhance the thermogenesis at brown adipose tissues in obese animals and to exert its anti-obese effect in part through such action. Therefore, BOF is thought to inhibit body weight gain in the present study not only by reducing food intake, but also by enhancing the thermogenesis. PIO attenuates compensatory hyperinsulinemia in type 2 diabetes by improving insulin sensitivity. In the present study, the serum insulin level of the PIO group tended to be lower than that of the control group after 1 week of treatment, although the difference was not significant. However, no differences in the serum insulin level were observed between the PIO group and the control group after 4 weeks of treatment, suggesting that the improvement in insulin sensitivity induced by PIO decreases as the treatment period lengthens. For this reason, the improvement in insulin sensitivity was thought to be canceled by the body weight gain after 4 weeks of PIO treatment. The serum insulin levels of the PIO+BOF4.3 and PIO+BOF6.4 groups also tended to be lower than that of the control group after 1 week of treatment. However, the serum insulin levels of these groups were not different from that of the control group after 4 weeks of treatment, similar to the situation in the PIO group. On the other hand, no differences in the serum insulin level were observed between the BOF6.4 group and the control group at either time point. This finding agrees with our previously reported results, suggesting that BOF itself has no effect on insulin sensitivity in KKA mice. Therefore, BOF was not thought to affect also the reduction in the PIO-induced improvement of insulin sensitivity.

An increase in adipose tissue mass is one of the causes of body weight gain induced by TZDs, since TZDs promote adipocyte differentiation through the activation of nuclear PPARγ. Regarding visceral fat, no differences in the retroperitoneal adipose tissue weights were observed among the groups in the present study. However, the epididymal adipose tissue weight of the PIO group tended to be lower than that of the control group. In contrast, the weight of the inguinal adipose tissue, a subcutaneous fat, of the PIO group tended to be higher than that of the control group. TZDs are known to shift the body fat distribution from visceral to subcutaneous regions, supporting the present results for PIO. However, the sum of these visceral and subcutaneous fat weights in the PIO group (3.60 g) was almost equal to that in the control group (3.48 g). On the other hand, the subcutaneous fat weights of the PIO+BOF4.3 and PIO+BOF6.4 groups tended to be lower than that of the PIO group. Such changes depended on the BOF dose and were similar to the changes in the serum glucose levels observed in these groups. These results suggest that the hypoglycemic effect of PIO is likely reduced in response to the increase in subcutaneous fat mass in KKA mice. In contrast, BOF may maintain the hypoglycemic effect of PIO by inhibiting the accumulation of subcutaneous fat, although it had no effect on fat weight by itself.

The livers of the control KKA mice were yellowish and the liver TG contents of the control KKA mice were higher than those of the normal mice. The liver TG content of the PIO group was markedly higher than that of the control group. In addition, the serum GPT level of the PIO group tended to be higher than that of the control group. These results suggest that PIO worsens fatty liver which occurs naturally in KKA mice. TZDs have been reported to cause severe fatty liver in KKA mice, supporting the results of the present study. On the other hand, the liver weights, TG contents and serum GPT levels of the PIO+BOF4.3 and PIO+BOF6.4 groups were lower than those of the PIO group in a manner that was dependent on the BOF dose.
Such changes were similar to the changes in the serum glucose levels observed in these groups. These results suggested that the worsening of the fatty liver might partly involve the reduction in the hypoglycemic effect of PIO. In contrast, BOF might maintain the hypoglycemic effect of PIO by preventing such worsening of fatty liver. This possibility is supported by our previous findings, in which BOF was shown to inhibit the increase in the liver TG content in fructose-loaded rats\(^{15}\) and ovariectomized rats.\(^{17}\)

Fluid retention is thought to be one of the causes of body weight gain induced by TZDs.\(^{24,25}\) Therefore, in the present study, the water intake and urine volume were determined after 4 weeks of treatment to investigate the state of body fluid. In diabetes, increases in water intake and urine volume as a result of hyperglycemia are generally observed. In the present study, the water intake and urine volume of the control KKA\(^{\nu}\) mice were also higher than those of the normal mice. The urine volume of the PIO group tended to be higher than that of the control group, although no differences in water intake were observed between these two groups. These results suggest that PIO might enhance fluid excretion and would not cause body weight gain as a result of fluid retention in KKA\(^{\nu}\) mice. On the other hand, both the water intake and urine volume of the PIO+BOF6.4 group were lower than not only those of the PIO group, but also those of the control group. A similar tendency was observed in the BOF6.4 group. The changes in water intake and urine volume in these BOF-treated groups were consistent with those in the serum glucose levels in these groups. Therefore, the decreases in water intake and urine volume in these BOF-treated groups are thought to be due to the attenuation of hyperglycemia.

In recent years, novel drugs for the treatment of type 2 diabetes that utilize incretin-related mechanisms have been developed, including receptor agonists of glucagon-like peptide-1 (GLP-1), a type of incretin, and inhibitors of dipeptidyl peptidase-4 (DPP-4), an incretin-degrading enzyme. These GLP-1 agonists and DPP-4 inhibitors reportedly do not cause body weight gains, unlike treatments with TZDs.\(^{26,27}\) However, considering the contribution of TZDs to the treatment of diabetes, the frequent use of TZDs is likely to continue. The present results suggest that the hypoglycemic effect of PIO could be maintained for a long time period by preventing body weight gain through a combined treatment involving PIO and BOF. This treatment strategy could benefit the long-term treatment of diabetes using PIO.

References


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