Effect of Estrogen Administration on Diabetic KK Mice
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KEY WORDS: estrogen, KK mouse.

KK mouse is well known strain of obese diabetes which resemble human maturity onset diabetes [7, 8, 11]. It has been reported that obese diabetes was induced easily by feeding a high caloric diet, or hyperphagia by gold-thioglucose administration in KK mice [8–10]. Monosodium aspartate (MSA) or monosodium glutamate (MSG) administrations in the neonatal period injured hypothalamic region and caused diabetes following obesity in KK mice [3, 12, 13]. The diabetes of KK mice is considered to be owing to disorder of lipid metabolism following obesity [2]. On the other hand, remarkable sex difference was observed in incidence of glycosuria in KK mice, the incidence of glycosuria in male was considerably higher than in female [2, 12, 14] and sex hormones were considered to play an important role in glycosuria appearance [5, 13]. Estrogen accelerates lipogenesis and diminishes plasma cholesterol level [17]. Estrogen administration is considered to have an influence on lipid metabolism.

In the present experiment, effect of estrogen administration was studied in the diabetic KK mice induced by MSA administration.

KK mice maintained in our laboratory were used in this experiment. The mice were fed with a commercial pellet, CMF (Oriental Yeast Co., Tokyo, Japan) ad libitum. Mice were administered subcutaneously with monosodium-L-aspartate (Wako Pure Chemical Industries, Osaka, Japan) in a dose of 4 mg/g body weight one day after birth. Urine glucose was tested by Tes Tape (Eli Lilly Co., Indianapolis, IN) weekly and urine which showed more than ++ (0.25%) in Tes Tape was identified as positive. Ten mice (♀:5, ♂:5) used as normal control were not treated with MSA, and without glycosuria at 30 weeks of age. In all mice administered with MSA, remarkable increase of food intake was not observed, but the body weights were 20 to 30% heavier than those of control mice at 12 weeks of age. The cumulative incidence of glycosuria to 30 weeks of age was over 90% and 30% in male and female mice, respectively. Glycosuric mice were divided to two groups, young (12 weeks old, ♀:6, ♂:6) and old (30 weeks old, ♀:8, ♂:6). The diabetic mice in both groups were administered intramuscularly with estradiol benzoate (Sankyo Zoki Co., Ltd., Tokyo, Japan) every day for 10 days in a dose of 50 μg/head and 20μg/head in male and female, respectively. Changes of glycosuria, levels of blood glucose, plasma insulin and FFA were measured. Blood glucose level was measured by glucose-oxidase method of Huggett and Nixon [6]. Plasma insulin level was measured by the micro ELISA sandwich method of Arai et al. [1]. Plasma FFA level was measured by a commercial kit, NEFA C Test Wako (Wako Pure Chemical Industries).

Effect of estrogen administration on glycosuria appearance is shown in Table 1. In all diabetic mice, glycosuria disappeared within 7 days after estrogen administration, and it was not detected for 2 months after estrogen administration in old group and female mice of young group. In male mice of young group, glycosuria began to appear again from the 5th day after stop of estrogen administration, and on the 39th day it was detected in all mice. Then estrogen was administered again, glycosuria disappeared within 2 days after the administration and it was not detected for 3 weeks. Changes of levels of blood glucose, plasma insulin and FFA in mice of old group are shown in Table 2. Before estrogen administration, blood glucose level was about 220 mg/dl, twice of normal level, and plasma FFA level was 3.6 to 4.4 mEq/l, 2 or 3 times higher than normal level. After estrogen administration, levels of blood glucose and plasma FFA decreased to normal levels. Plasma insulin level decreased to 116 to 120 μU/ml. Changes of blood glucose and plasma FFA levels in young male mice are shown in Fig. 1. The average of blood glucose level was about 220 mg/dl before estrogen administration, and it decreased to 100 mg/dl on the 10th day. However, glycosuria appeared again and blood glucose level increased to 210 mg/dl after stop of
the estrogen administration. On the 39th day, estrogen was administered again, and glycosuria disappeared immediately. The average blood glucose level decreased to 105 mg/dl 2 days after the administration. Plasma FFA level showed the similar changing pattern of blood glucose level.

Considerable difference in sexes has been reported in the incidence of glycosuria in KK mice [2, 12, 14], and in the diabetic male KK mice, glycosuria disappeared immediately after castration which eliminated the effect of testosterone [13]. In the young (under 20 weeks old) diabetic male KK mice, glycosuria disappeared temporarily after castration, but it appeared again soon. Recurrence of glycosuria was considered to be owing to the effect of dehydroyepiandrosterone (DHA) as adrenal androgen [5, 13]. Sex hormones are considered to have an important role in onset of diabetes in KK mice. In this experiment, over dosage of estrogen administration was tried in diabetic KK mice of two groups, young and old, instead of castration. Estrogen administration was very effective to improve the diabetic conditions in KK mice. Plasma FFA level decreased remarkably with estrogen administration. Glycosuria was detected in KK mice with high plasma FFA level, over 3.5 mEq/l, and it disappeared when plasma FFA level decreased.

Table 1. Effect of estrogen administration\(^a\) on glycosuria appearance in diabetic KK mice

<table>
<thead>
<tr>
<th>Glycosuria appearance (glycosuric mice/mice examined)</th>
<th>0(^b)</th>
<th>10</th>
<th>39</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old group (30 weeks old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Female</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Young group (12 weeks old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male(^c)</td>
<td>6/6</td>
<td>0/6</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Female</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

\(a\) Estrogen was administered intramuscularly every day for ten days in a dose of 50 µg/head in male, 20 µg/head in female.
\(b\) Numbers mean days after first estrogen administration.
\(c\) Male mice of young group were administered with estrogen in a dose of 50 µg/head on the 39th day.

Table 2. Effect of estrogen administration in the diabetic old KK mice\(^a\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glycosuria</th>
<th>Blood glucose (mg/dl)</th>
<th>Plasma insulin (µU/ml)</th>
<th>Plasma FFA (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n=8)</td>
<td>+ + + (^b)</td>
<td>210±20 (^c)</td>
<td>160±18</td>
<td>4.4±0.8</td>
</tr>
<tr>
<td>Pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10th day of estrogen treatment</td>
<td>—</td>
<td>90±15</td>
<td>116±10</td>
<td>1.6±0.6</td>
</tr>
<tr>
<td>Control(^d) (n=5)</td>
<td></td>
<td>105±10</td>
<td>74±8</td>
<td>1.4±0.6</td>
</tr>
<tr>
<td>Female (n=6)</td>
<td>+ +</td>
<td>225±20</td>
<td>150±22</td>
<td>3.6±1.2</td>
</tr>
<tr>
<td>Pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10th day of estrogen treatment</td>
<td>—</td>
<td>120±15</td>
<td>120±18</td>
<td>1.2±0.5</td>
</tr>
<tr>
<td>Control(^d) (n=5)</td>
<td></td>
<td>112±14</td>
<td>86±10</td>
<td>1.3±0.5</td>
</tr>
</tbody>
</table>

\(a\) 30 weeks old. \(b\) 0.5% by Tes Tape. \(c\) means ± S.D.  
\(d\) without MSA administration.
to below 2.0 mEq/l. Disappearance of glycosuria in diabetic KK mice was considered to be owing to decrease of plasma FFA level by estrogen administration. Recurrence of glycosuria observed in young male mice was considered to be owing to more DHA secreted from adrenal cortex than in old mice. However, secreting mechanism or relation to estrogen are different between testosterone and adrenal androgen [15], and effect of androgen on lipid metabolism needs further investigation.

On the other hand, plasma insulin level was considerably high, over 100 μU/ml, in young KK mice without glycosuria. Glucose tolerance is impaired in KK mice because of hepatic low glycolytic and high lipogenic abilities [4, 16]. And peripheral insulin sensitivity of KK mice is diminished and insulin receptors of liver plasma membranes decreased. These characters related with the insulin resistance present in KK mice [4, 7]. Relationships between hyperinsulinism and obesity or changes of plasma levels of lipid except FFA in KK mice should be studied.

It is suggested that improvement of metabolic disorder of lipid is very important for curing of diabetes in KK mice. However, effect of endocrine abnormalities by MSA on glycosuria or effect of estrogen administration on other diabetic animals should be studied.

REFERENCES


Fig. 1. Effect of estrogen administration in diabetic young male KK mice (12 weeks old). Blood glucose and plasma FFA levels are given in means ± S. D. Arrows indicate estrogen administration in a dose of 50 μg/head.

要約
糖尿病 KK マウスにおけるエストロジェン投与の効果（短報）：新井敏郎・富岡玲子・大木与志雄（日本獣医畜産大学獣医生理化学教室）——MSA 投与により誘発された肥満性糖尿病 KK マウスを若齢、老齢の 2 群に分けエストロジェン投与の治療効果を調べた。エストロジェン投与により血中遊離脂肪酸が減少するのに伴い、血糖値が低下し尿糖が消失した。若齢群マウスでは投与中止後、尿糖が再び出現したが、エストロジェン再投与により尿糖は直ちに消失した。KK マウスの肥満性糖尿病の治療にエストロジェンが有効であることが明らかとなった。