Renal Anemia in Polycystic Kidney Disease Mouse

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ABSTRACT. DBA/2FG-pcy mice developed the chronic renal failure by the progressive polycystic formation at five months old. Their bilaterally enlarged kidneys occupied about 15% of the body weight. It was about 9 times larger than the normal kidney of DBA/2N mice. A large number of various-sized cysts appeared in cortex and medulla of bilateral light-yellow kidneys of sponge-like shape. Blood urea nitrogen and creatinine increased. Red blood cells (746±39 × 10^6/mm^3), hemoglobin (8.8±0.4 g/dl) and hematocrits (31.1±1.5%) were lower than those of normal control mice. Serum erythropoietin level and reticulocyte did not increase. In addition, the treatment with exogenous erythropoietin improved the anemia in DBA/2FG-pcy mice. It was suggested that the anemia in DBA/2FG-pcy mice was due to the disorder of erythropoietin production caused by the progressive polycystic formation in kidneys.—Key words: erythropoietin, mouse, polycystic kidney, spontaneous renal anemia


Polycystic kidney disease was considered to be due to a congenital growth abnormality and was known as one of the hereditary kidney deformations [20]. The polycystic kidney disease mouse was found by Takahashi et al. as a mutant in the KK strain. The pcy gene for polycystic kidney disease was confirmed by the cross-mating genetic analysis [21]. DBA/2FG-pcy mouse inherited the pcy gene and showed genetical polycystic kidney disease. Their symptoms resembled those of human adult type polycystic kidney disease (Potter's type III) in clinical course and morphological features [18].

The purpose of this study is to elucidate the characteristics of the anemia induced by the progress of polycystic kidney disease in DBA/2FG-pcy mice and to examine the usefulness of this strain of mice as a spontaneous renal anemic model. To clarify the nature of this anemia, both endogenous serum erythropoietin titers and reticulocyte counts of these mice were compared with those of other anemic mice, and the hemopoietic response to the treatment of exogenous erythropoietin was also examined in DBA/2FG-pcy mice.

MATERIALS AND METHODS

Polycystic kidney disease mouse: In 1983, the fertilized ova were collected from polycystic kidney disease mouse of DBA/2FG-pcy which were obtained from the laboratory animal center, Institute for Comprehensive Medical Science, School of Medicine, Fujita-Gakuen University in Japan. They were stored frozen for 5 years. DBA/2FG-pcy mice were bred in July, 1988 by transplanting the fertilized ova to female mice of ICR strain [6]. The mice were kept at 23±2°C, and humidity of 50±10%. The standard commercial diet (CRF-1, Oriental-Yeast Co., Ltd., Japan) and tap water were given to the mice ad libitum. For the following experiment, fifty-three DBA/2FG-pcy mice (male; 22, female; 31) around 5 months old were used.

Other mice: As the other control mice, 5-month-old DBA/2N (Charles River Japan Inc., Japan) were used. Normal control mice were not treated at all. Hemolytic anemia models were prepared by injecting 60 mg of phenylhydrazine per kg of body weight intraperitoneally once a day for 3 days. Bleedened anemia models were prepared by bleeding 100 μl of blood per mouse from orbital sinus once a day for 3 days.

Hematological and clinico biochemical examination: Mice were sacrificed by ether and blood were collected from caudal vena cava. Red blood cell counts (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were analyzed with auto-cell counter (E-4,000, Sysmex, Japan). Reticulocyte counts (RET) were measured by a method of Brecher [1]. Blood urea nitrogen (BUN), creatinine (CRE), serum iron (Fe) and total serum protein (TP) were analyzed with automatic serum analyzer (model 7,150, Hitachi, Japan). Serum erythropoietin (EPO) was measured by the
enzyme immunoassay [4].

Pathological examination: At the end of examinations, gross lesions of all the mice were observed. After their kidneys were weighed, they were fixed in 10% formalin. Paraffin sections were stained with hematoxylin and eosin. Kidneys were observed with a microscope.

Administration schedule of erythropoietin to DBA/2FG-pcy mice: In order to uniform the anemic conditions among groups, HCT of all mice were measured by the microcapillary method before dividing mice to each group. To investigate the improvement of anemia with recombinant human erythropoietin (rhEPO) [3, 4], each group of 5 DBA/2FG-pcy mice was respectively injected with 50, 250, 1,250, or 5,000 unit of rhEPO per kg of body weight 5 times for 5 days. Hematological examinations were carried out 5 days after the last injection. Moreover, in order to know the progress of erythropoiesis, RET and serum EPO levels were examined in other DBA/2FG-pcy mice 16 hrs after the 4th injection. These values both 16 hrs after the 4th injection and 5 days after the last injection were compared.

Statistical analysis: Significant differences among groups were analyzed by the analysis of one-way layout variance and Scheffe's multiple comparison. Differences between normal control mice and DBA/2FG-pcy mice were tested using Student's t-test. Mono-regression analysis was used for reticulocyte counts and serum EPO titers.

RESULTS

Clinical appearance: All of the DBA/2FG-pcy mice became noticeable by the enlargement of bilateral kidneys by a palpation. They showed poor behavior with significantly lower body weight compared with normal DBA/2N mice (Table 1).

Pathological appearance: Pathological abnormality was observed in the kidneys of DBA/2FG-pcy mice. A large number of various-sized cysts appeared in cortex and medulla of bilateral light-yellow kidneys of sponge-like shape (Fig. 1). Kidneys were grossly enlarged and their weight became 9 times heavier than those of normal mice, occupying about 15% of the body weight (Table 1). Histologically, innumerable watery cysts were observed in cortex, and extended tube cavities were lined by flat simple epithelium. At the epithelium of remained small ureter, bladder often appeared. Renal corpuscle showed resistence morphologically against the abnormal changes of the circumference. Interstitium increased slightly (Fig. 1).

Hematological appearance and serum chemistry: BUN (113.7±8.8 mg/dl) and CRE (0.75±0.03 mg/dl) were respectively 4-fold and 2-fold higher than those of normal control mice (Table 1).

RBC (746±39 × 10^6/mm^3), HGB (8.8±0.4 g/dl) and HCT (31.1±1.5%) were lower than those of normal control mice. Lower values of MCV, MCH and MCHC were also observed (Table 2). Characteristics of the anemia were compared to those of both bleded anemic mice and hemolytic anemic mice. Similarly to other anemic mice, DBA/2FG-pcy mice showed a marked decrease in HCT compared with normal control mice. Although the elevation of serum EPO titers and RET were observed both in bleded anemic mice and in hemolytic anemic mice, those findings were not observed in DBA/2FG-pcy mice similarly to the normal control mice. Serum iron (Fe) decreased in DBA/2FG-pcy mice (Table 3).

Hemopoietic response induced by rhEPO: RET and serum EPO titers 16 hrs after the 4th injection of rhEPO were examined. RET increased dose-

<table>
<thead>
<tr>
<th>Table 1. Kidney weight, blood urea nitrogen (BUN) and creatinine in the polycystic kidney disease mice</th>
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<tr>
<td>Body weight (g)</td>
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<td>Kidney weight (R) mg/g</td>
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<td>(L) mg/g</td>
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<tr>
<td>Total protein (g/dl)</td>
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<td>BUN (mg/dl)</td>
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<td>Creatinine (mg/dl)</td>
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a) Significant difference from normal mice at P<0.01.
b) Numbers of animals.
c) Each value represents the mean±S.E.
Fig. 1. (a) Comparison photograph shows the difference in size of the polycystic kidneys of DBA/2FG-pcy mice (left) from the normal kidneys of DBA/2N mice (right).
(b) Light microscopic features of the polycystic kidneys of DBA/2FG-pcy mice at the age of 5 months. Many markedly dilated cysts are observed (hematoxylin & eosin, ×5).
(c) Light microscopic features of the normal kidneys of DBA/2N mice at the age of 5 months (hematoxylin & eosin, ×5).
Table 2. Comparison of hematological parameters in the polycystic kidney disease mice with the normal mice

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<tr>
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<th>Polycystic kidney mice (DBA/2FG-pcy)</th>
<th>Normal control mice (DBA/2N)</th>
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<tbody>
<tr>
<td>Numbers of animals</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Red blood cell (×10^6/mm^3)</td>
<td>746±39.1***</td>
<td>1002±8.8^b</td>
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<td>Hemoglobin (g/dl)</td>
<td>8.8±0.43**</td>
<td>14.0±0.09</td>
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<td>Hematocrit (%)</td>
<td>31.1±1.50**</td>
<td>47.7±0.34</td>
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<td>MCV (fl)</td>
<td>41.8±0.36**</td>
<td>43.7±0.13</td>
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<td>MCH (pg)</td>
<td>11.8±0.10**</td>
<td>12.9±0.05</td>
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<td>MCHC (%)</td>
<td>28.2±0.23**</td>
<td>29.4±0.11</td>
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<td>Platelet (×10^9/mm^3)</td>
<td>151±16.6*</td>
<td>95±2.0</td>
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<tr>
<td>White blood cell (×10^3/mm^3)</td>
<td>35±4.4</td>
<td>43±1.6</td>
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a) Significant difference from normal mice at P<0.01.
b) Each value represents the mean ±S.E.

dependently with rhEPO. These increases correlated with the serum EPO titers. Five days after the last injection of rhEPO, RET tended to decrease compared with the value 16 hrs after the 4th injection. Serum EPO titers decreased to reach the normal range 5 days after the last injection (Fig. 2).

Five repetitive EPO injections improved the anemia of DBA/2FG-pcy mice. These hematological parameters were increased dose-dependently by the rhEPO injections. Both HCT and RBC were improved to the level of normal range by the subcutaneous injections with rhEPO of more than 1,250 U/kg. On the other hand, HGB were apparently increased by the rhEPO injections, but the values did not recover to the level of normal range (Fig. 3).

DISCUSSION

The progress of polycystic kidney disease induced the renal anemia in DBA/2FG-pcy mice. W/W^v mice and S/S^d mice are known as the myeloplastic anemic models and NZB mice are known as the model of hemolytic anemia due to the autoim-
mune disease [7, 8, 19]. However, the spontaneous renal anemic model has never been reported.

In 5-month-old DBA/2FG-pcy mice, bilaterally enlarged kidneys occupied about 15% of the body weight, approximately 9 times heavier than those of normal mice. They showed a light-yellow sponge-like shape with a large number of various-sized cysts appeared in cortex and medulla. Histologically, innumerable watery cysts were observed in cortex and extended tube cavities were lined by flat simple epithelium. These pathological findings consisted with the view of the mutant polycystic kidney mouse which had been reported by Takahashi et al. [21]. BUN and CRE of DBA/2FG-pcy mice were 4-fold and 2-fold higher than those of normal control mice, respectively. These results demonstrated that 5-month-old DBA/2FG-pcy mice fell chronic renal failure.

In 5-month-old DBA/2FG-pcy mice, RBC, HGB and HCT were lower than those of normal control mice. Lower values of MCV, MCH, MCHC and serum iron were also recorded. DBA/2FG-pcy mice showed severe anemic states identical to both bleeded and hemolytic anemic mice. The elevation
of serum EPO titers and RET were observed both in bleeded and hemolytic anemic mice, but not in DBA/2FG-pcy mice. Endogenous serum EPO titers and RET did not increase identical to those of renal anemic model suffered with a surgical removal of the kidney to decline an appropriate production of endogenous erythropoietin [11].

We have reported that the renal anemic model, successfully prepared by the surgical nephrectomy, was improved by the treatment of exogenous erythropoietin [9, 10]. In the present study, the hemopoietic response induced by the treatment of exogenous erythropoietin on DBA/2FG-pcy mice was also examined. Treatment of exogenous erythropoietin remarkably improved the anemia of DBA/2FG-pcy mice. Reticulocyte counts increased dose-dependently 16 hrs after the 4th injection of rhEPO. These increases correlated with the serum EPO titers. Hematological parameters of HCT, RBC and HGB were increased dose-dependently 5 days after the 5 repetitive rhEPO injections. All these results provided that DBA/2FG-pcy mice showed the reactive anemia with erythropoietin injections similar to the anemia induced by partial nephrectomy [12]. This severe anemic state was considered to be due to the disorder of kidney function by the progressive polycystic formation [2, 17].

Both HCT and RBC were improved to the normal range by the rhEPO injections of more than 1,250 U/kg. On the other hand, HGB were apparently increased by the rhEPO injections, but the values were not within the normal range. The role of inhibitors of erythropoiesis in anemia of chronic renal failure is not completely clarified. However, substances that inhibit growth of erythroid progenitors, heme synthesis, and DNA synthesis in cultured bone marrow have been reported [16, 15, 5]. It has also been demonstrated that uremic sera reduce the biological activity of erythropoietin and that serum
iron decrease in uremic anemia [13, 14, 2]. Although uremic toxin is not a major contributing factor in the renal anemia [17], the present study indicated that it influenced the erythropoiesis induced by the rhEPO on DBA/2FG-pcy mice. It is suggested that DBA/2FG-pcy mice are useful as a spontaneous renal anemic model.

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REFERENCES