Effect of Human Erythropoietin (hEPO) Treatment on Anemia in ICR-derived Glomerulonephritis (ICGN) Mice

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Abstract: ICR-derived glomerulonephritis (ICGN) mice are a novel inbred strain with hereditary nephrotic syndrome and are thus considered a good animal model of human idiopathic nephrotic syndrome. In the present study, we investigated the effect to erythrocyte production by human erythropoietin (hEPO) treatment in ICGN mice during the early nephrotic stage. Erythrocyte count, hemoglobin concentration and hematocrit value in hEPO-treated (5 U/body/day, for 5 days) ICGN mice were recovered to the levels found in normal ICR mice. In addition, there was no correlation between plasma creatinine level, a marker of renal function, and erythrocyte count after hEPO treatment. Therefore, anemia in ICGN mice may be caused by decreased production of EPO in the kidney following progressive parenchymal damage.

Key words: anemia, human erythropoietin (hEPO), ICR-derived glomerulonephritis (ICGN) mice, renal parenchymal damage

Introduction

ICR-derived glomerulonephritis (ICGN) mice are a novel inbred strain with a hereditary nephrotic syndrome and are considered a good animal model of human idiopathic nephrotic syndrome [1] or nephritis [3, 4, 16]. ICGN mice show proteinuria at a young age, later develop hypoproteinemia, hyperlipidemia, anemia, and systemic edema [10, 11, 14]. Previous studies by Ogura

et al. [10, 11] and Yamaguchi *et al.* [15] showed that ICGN mice might develop severe anemia because of progressive renal parenchymal damages. It was demonstrated that peritubular cells are renal erythropoietin-producing cells [2, 5]. Severe anemia in ICGN mice is related to the regression of erythropoietin-producing cells [8, 9, 12, 13]. Unknown tubulointerstitial cells undergoing apoptosis were similar to renal erythropoietin-producing cells detected by

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in situ hybridization for erythropoietin mRNA [8, 9], and could be identified as these cells [15]. In the present study, we investigated the effect of the human erythropoietin (hEPO) treatment on the erythrocyte count in 10-week old ICGN mice.

Materials and Methods

Animals and treatments

ICGN mice from the National Institute of Infectious Diseases (NIID, Tokyo, Japan) were prepared by mating homozygous males (*nep/nep*) and heterozygous females (*nep/-*) at Kyoto University (Kyoto, Japan). The animals were housed in autoclaved metal cages and were given a standard diet (CM: Oriental Yeast, Japan) and tap water *ad libitum* in an air-conditioned room ($23 \pm 1^\circ\text{C}$) under controlled lighting conditions (12 h light/12 h dark). In this experiment, ten ICGN male mice that exhibited proteinuria were used at 10 weeks of age. Five ICGN mice were injected subcutaneously with hEPO (Sigma, USA) at a dose of 5 U/body/day in saline for 5 days, and the other five male mice were not treated and used as control.

Hematology

Hematological parameters were determined in plasma samples from blood obtained from the heart under anesthesia with pentobarbital sodium 1 day after the final hEPO treatment. An Automated Hematology Analyzer K-4500 (Sysmex Co., Japan) was used to determine leukocyte counts (WBC), erythrocyte counts (RBC), platelet counts (PLT), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hematocrit (MCH) and mean corpuscular hematocrit concentration (MCHC).

Blood chemistry

Blood chemical markers were determined in plasma samples from blood obtained from the heart under anesthesia with pentobarbital sodium 1 day after the final hEPO treatment. An Automatic Analyzer 7070 (Hitachi Instruments Service Co., Ltd, Japan) and Autosera kits were used to determine the total protein (burette method), albumin (BCG method), glucose (hexokinase method), BUN (urease-GLDH method), and creatinine (Jaffe's method) levels.

Histopathology

The mice were euthanized 1 day after the final hEPO treatment. Then the kidneys and femur were quickly removed and fixed in 10% buffered formalin. After fixation, the femur was immersed in 10% buffered EDTA for decalcification, and all organs were rinsed in tap water, dehydrated in a graded alcohol series, embedded in paraffin, and cut into sections 5- μm thick. Cross-sections were used for hematoxylin-eosin staining. Histopathological changes were observed under a light microscope, and graded as no remarkable change, slight, moderate or marked.

Statistical analysis

All measured values were analyzed by one-way ANOVA and comparison by Student's t-test or Aspin-Welch's t-test. The correlation between RBC and creatinine levels was calculated by regression analysis.

Results

Hematology and blood chemistry

Ht was significantly increased in the hEPO-treated mice, and the RBC and Hb were slightly increased compared with those in the control (Table 1). Other parameters (WBC, MCV, MCH, MCHC, PLT) did not differ in the hEPO-treated mice (Table 1).

The total protein level was significantly increased in the hEPO-treated mice, and the albumin level was more increased compared with the control (Table 2). However, the BUN and glucose levels were less than those in the control (Table 2). The creatinine level was not changed in the hEPO-treated mice.

The correlation coefficient ($r=0.040435$) between the RBC value and the creatinine level was not significant ($p=0.189$).

Histopathology

Glomerulosclerosis, basophilic tubules, hyalin casts in tubules, interstitial fibrosis and interstitial lymphocyte infiltration of the kidneys were observed in the hEPO-treated mice and controls, with no difference between the two groups (Table 3).

There was no remarkable histological difference of the bone marrow in the femur between the two groups (Table 3).

Table 1. Hematological changes in ICGN mice treated with hEPO

Parameter	Control ^{a)}	hEPO Treatment ^{b)}
WBC (10 ² /ml)	44.7 ± 16.0 (3)	33.0 ± 8.5 (4)
RBC (10 ⁴ /ml)	729.3 ± 89.5 (3)	833.0 ± 82.4 (4)
Hb (g/dl)	12.6 ± 1.7 (3)	15.4 ± 1.5 (4)
Ht (%)	41.2 ± 5.7 (3)	51.6 ± 4.8 (4)*
PLT (10 ⁴ /ml)	129.1 ± 20.9 (3)	109.3 ± 15.3 (4)
MCV (fl)	56.5 ± 0.9 (3)	62.0 ± 0.8 (4)
MCH (pg)	17.2 ± 0.2 (3)	18.5 ± 0.3 (4)
MCHC (g/dl)	30.6 ± 0.2 (3)	29.9 ± 0.1 (4)

Values are means ± S.D. for the numbers of mice indicated in parentheses. Significantly different from controls, *: p<0.05.

^{a)}Plasma volumes of two mice were not enough to measure.

^{b)}Plasma volume of one mouse was not enough to measure.

Table 2. Blood chemical changes in ICGN mice treated with hEPO

Marker	Control ^{a)}	hEPO Treatment
TP (g/dl)	3.50 ± 0.51 (4)	4.28 ± 0.34 (5)*
Alb (g/dl)	1.12 ± 0.20 (4)	1.35 ± 0.10 (5)
BUN (mg/dl)	29.0 ± 10.2 (4)	25.5 ± 4.0 (5)
Cre (mg/dl)	0.35 ± 0.05 (4)	0.35 ± 0.03 (5)
Glc (mg/dl)	179.0 ± 25.5 (4)	157.2 ± 17.3 (5)

Values are means ± S.D. for the numbers of mice indicated in parentheses. Significantly different from controls, *: p<0.05.

^{a)}Plasma volume of one mouse was not enough to measure.

Table 3. Histopathological changes in ICGN mice treated with hEPO

Organs and Findings	Grade ^{a)}	Control (5)	hEPO Treatment (5)
Kidney	NR	0	0
Basophilic tubule	1	3	2
	2	0	0
	3	1	0
Hyalin cast in tubule	1	1	0
	2	0	0
	3	0	0
Interstitial fibrosis	1	4	4
	2	0	0
	3	0	0
Interstitial lymphocyte infiltration	1	3	2
	2	0	0
	3	0	0
Glomerulosclerosis	1	1	2
	2	3	3
	3	1	0
Bone marrow	NR	5	5

^{a)}Grade of change: NR, not remarkable changes; 1, slight; 2, moderate; 3, marked.

Discussion

The effect of the hEPO treatment on anemia was investigated in 10-week old ICGN mice.

The RBC, Hb and Ht values in control ICGN mice were lower than those in normal ICR mice of the same age (in-house data: RBC, 882.0 ± 55.2; Hb, 14.57 ± 0.88; Ht, 48.71 ± 2.61). The MCV value which exhibits hemoconcentration, was not significantly changed after the hEPO treatment. We suggest that ICGN mice

showed renal anemia as reported by Ogura *et al.* [10, 11] and Yamaguchi-Yamada *et al.* [15]. RBC, Hb and Ht values were increased in the hEPO-treated mice and were similar to the levels found in normal ICR mice. These results indicate that the hEPO treatment improved anemia in the ICGN mice by inducing proliferation of erythrocytes. Renal anemia in ICGN mice may be induced by a decrease in endogenous erythropoietin synthesis. It was reported that the sites of EPO synthesis were mainly peritubular capillary interstitial cells in

the kidneys [2, 5, 7]. Since the major biological effects of EPO promote the proliferation or differentiation of proerythroblasts and inhibit apoptosis of erythroblasts [6, 9], hEPO treatment may improve anemia in ICGN mice. The increased thickness of the fused glomerular basement membrane (GBM) by excessive accumulation of basement membrane material secreted from epithelial cells and subsequent following overall loss of epithelial foot processes in the glomeruli in newborn ICGN mice indicates that the nephrotic disease in ICGN mice is caused by some defects in the GBM maturation process, which may be crucial for the generation of glomerular permselectivity [10]. In the present study, we observed some histopathological findings such as interstitial fibrosis, glomerulosclerosis and hyaline casts in control and hEPO-treated mice similar to the findings in previous studies [10, 11]. This spontaneous disease is similar to human nephrotic syndrome caused by idiopathic glomerular lesions [10]. There was no difference in these renal lesions between control mice and hEPO-treated ones showing amelioration of anemia. In addition, there was no correlation between the creatinine level and the RBC value. These findings suggest that hEPO may not improve renal injury but directly induces erythropoiesis in ICGN mice. Therefore, anemia may be caused by decreased production of EPO following the progression of parenchymal damage in ICGN mice.

References

1. Abramowsky, C.R., Aikawa, M., Swinehart, G.L., and Snajdar, R.M. 1984. Spontaneous nephrotic syndrome in a genetic rat model. *Am. J. Pathol.* 117: 400–408.
2. Bunn, H.F. and Poyton, R.O. 1996. Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* 76: 839–885.
3. Cosgrove, D., Meehan, D.T., Grunkemeyer, J.A., Kornak, J.M., Sayers, R., Hunter, W.J., and Samuelson, G.C. 1996. Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. *Genes Dev.* 10: 2981–2992.
4. Jansen, B., Thorner, P.S., Singh, A., Patterson, J.M., Lumsden, J.H., Valli, V.E., Baumal, R., and Basur, R.K. 1984. Animal model of human disease: hereditary nephritis in Samoyed dogs. *Am. J. Pathol.* 116: 175–178.
5. Jelkmann, W. 1992. Erythropoietin: structure, control of production, and function. *Physiol. Rev.* 72: 449–489.
6. Koury, M.J. and Bondurant, M.C. 1990. Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells. *Science* 248: 378–381.
7. Lacombe, C., Da Silva, J.L., Bruneval, P., Fournier, J.G., Wendling, F., Casadevall, N., Camilleri, J.P., Bariety, J., Varet, B., and Tambourin, P. 1988. Peritubular cells are the sites of erythropoietin synthesis in the murine hypoxic kidney. *J. Clin. Invest.* 81: 620–623.
8. Maxwell, P.H., Ferguson, D.J., Nicholls, L.G., Iredale, J.P., Pugh, C.W., Johnson, M.H., and Ratcliffe, P.J. 1997. Sites of erythropoietin production. *Kidney Int.* 51: 393–401.
9. Muta, K. and Krantz, S.B. 1993. Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin. *J. Cell Physiol.* 156: 264–271.
10. Ogura, A., Asano, T., Matsuda, J., Takano, K., Nakagawa, M., and Fukui, M. 1989. Characteristics of mutant mice (ICGN) with spontaneous renal lesions: a new model for human nephrotic syndrome. *Lab. Anim.* 23: 169–174.
11. Ogura, A., Asano, T., Suzuki, O., Yamamoto, Y., Noguchi, Y., Kawaguchi, H., and Yamaguchi, Y. 1994. Hereditary nephrotic syndrome with progression to renal failure in a mouse model (ICGN strain): clinical study. *Nephron* 68: 239–244.
12. Schuster, S.J., Wilson, J.H., Erslev, A.J., and Caro, J. 1987. Physiologic regulation and tissue localization of renal erythropoietin messenger RNA. *Blood*. 70: 316–318.
13. Silva, J.L., Schwartzman, M.L., Goodman, A., Levere, R.D., and Abraham, N.G. 1994. Localization of erythropoietin mRNA in the rat kidney by polymerase chain reaction. *J. Cell Biochem.* 54: 239–246.
14. Tatsumi, H., Satoh, S., Okamoto, M., Nakamura, M., Asano, T., and Kurosawa, T. 1995. Morphological studies on the kidney of the spontaneous nephrotic (ICGN) mice in the late stage. *Acta. Anat. Nipp.* 70: 96–106.
15. Yamaguchi, M., Manabe, N., Uchio-Yamada, K., Akashi, N., Yamamoto, Y., Ogura, A., and Miyamoto, H. 2001. Localization of proliferative and apoptotic cells in the kidneys of ICR-derived glomerulonephritis (ICGN) mice. *J. Vet. Med. Sci.* 63: 781–787.
16. Zheng, K., Thorner, P.S., Marrano, P., Baumal, R., and McInnes, R.R. 1994. Canine X chromosome-linked hereditary nephritis: a genetic model for human X-linked hereditary nephritis resulting from a single base mutation in the gene encoding the alpha 5 chain of collagen type IV. *Proc. Natl. Acad. Sci. U.S.A.* 91: 3989–3993.