SYMPOSIUM (II)

PATHOGENESIS AND MANAGEMENT OF RENAL ANEMIA

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A, PATHOGENESIS

STUDIES ON MECHANISM OF ANEMIA ASSOCIATED WITH RENAL FAILURE, WITH SPECIAL REFERENCE TO ERYTHROPOIETIN.

by

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Introduction:

It has been well documented that the circulating red blood mass is maintained at constant level through a dynamic equilibrium between production and destruction. The intractable anemia associated with chronic renal failure is undoubtedly caused by impairment of the above two processes. The association of anemia would evidently reduce the effective renal blood blow, resulting in a marked suppression of the kidney function. For these reasons, to elucidate the pathogenesis of renal anemia, studies on the individual factors causing anemia, as well as on the combined effects are required.

Erythropoietin (Epo), being regarded to regulate primarily the rate of erythropoiesis, is considered to have a close relationship with the kidney as the possible main production site.
In the present paper, our recent studies on the mechanism of renal anemia were reported with special reference to Epo.

**Kidney and Erythropoietin:**

In the following studies, in vivo uptake rate of radioactive iron (Fe^{59}) into the peripheral blood of the starved rats and of the hypoxia-induced polycythemic mice was used as the bioassay method of Epo.

Exposure to hypoxia of 1/2 atmospheric pressure in an anoxic chamber caused an increased production of Epo in the intact rats, followed by a gradual increase of reticulocyte counts and hematocrit values after prolonged exposure. Plasma Epo activity, detected within first 3 hrs. of exposure, was further increased until a peak was achieved on the 16th hr. of hypoxia. A group of uremic rats caused by bilateral ureter ligation showed a same response of Epo as in normal rats. On the other hand, Epo was not detected in the another group of uremic rats by bilateral nephrectomy even after 16 hrs. of hypoxia.

It was thus demonstrated that a presence of the kidney is essential for Epo production.

In this respect, rats with nephrotoxin induced nephritis were examined. Two sorts of nephrotoxins were obtained according to the method previously described by Shibata et al, and two groups of rats were injected with these nephrotoxins. Following 16 hrs. of hypoxia, Epo levels of the plasma from 2 groups of the nephritic rats were significantly lower than that of normal hypoxic rats.

Above experimental results indicate that the competence for producing Epo in response to such

| Table 1. Plasma Epo. Level after Anoxia (Polycythemic Mouse Assay) |
|--------------------------|-----------------------|-------------------|
|                          | No. of Mice | Hct. | %Fe^{59} Incorporation Mean ± S.E. |
| Saline                   | 8           | 63   | 0.31 ± 0.06                        |
| Nephritis A              | 5           | 60   | 11.64 ± 1.19                       |
| Nephritis B              | 5           | 59   | 13.50 ± 1.61                       |
| Control (Untreated)      | 5           | 59   | 20.15 ± 1.04                       |
a treatment as exposure to low pressure was diminished, when the organic and possibly the functional disturbance of the kidney was present.

**Renal Anemia and Erythropoietin (Clinical Investigations):**

Plasma Epo levels of anemic patients were assayed. Other than the patients with aplastic anemia and renal insufficiency, the plasma Epo level showed a negative correlation with the degree of anemia, and a statistically significant regression line was obtained. Plasma Epo titres of 17 samples from 13 uremic patients were plotted under the area of the statistical line as shown in Fig. 1. This result would lead to a conclusion that the expected elevation of Epo production can not be achieved in the cases of uremic anemic patients. To exclude the possibility of the presence of Epo antagonist in the uremic plasma, urinary Epo with known activity was divided into two incubating tubes, each containing 5 ml. of normal and uremic human plasma respectively. Immediately after incubation for 30 min. at 37°C, pH 7.4, their Epo activities were assayed. No significant difference was detected between the two samples thus treated.

Recently, renal homotransplantation has been successfully tried. In this report, 5 cases of uremia were examined before and after renal transplantation in respect to their erythropoiesis.

Following transplantation, marked increase in reticulocyte counts, shortening of plasma iron disappearance (T/2) and an increased red cell iron utilization (%) were observed in all cases studied.
These results show an improvement of erythropoiesis in the subjects treated with the kidney transplantation. To elucidate the mechanism of this improvement, plasma Epo titre was assayed. Most of the plasmas obtained from the patients after transplantation showed elevated Epo activities. These results are also considered to indicate that the transplanted kidney has a capacity to produce Epo.

Based on the above described results, a therapeutic usefulness of Epo on the uremic anemia was then clinically examined. The Epo used was obtained from the urine of patients with aplastic anemia, and partial by purified.

Reticulocytosis as well as marrow erythroid hyperlasia was observed after administration of Epo in a hematologically normal and also in a severe uremic patient.

Above clinical results are seemed to indicate that the uremic bone marrow still contains the Epo responsible stem cells. This presence of the stem cells in the uremic bone marrow was further proved by the following in vitro tissue culture method.

As shown in Fig. 2, the uremic bone marrows showed an erythropoietic response according to the dosis of added Epo. There was no difference in the degree of the response between normal and uremic marrows.

Fig. 2 Effect of erythropoietin on marrow cells from uremic patients
Mechanism of Erythropoietin Production:

In next series of experiments dealing with the extraction of erythropoietic substance from various organs, existence of the precursor and the activator of Epo was suggested, but the details of these findings will be reported elsewhere.

Summary:

In this paper, our clinical and experimental studies on mechanism of renal anemia, in which Epo plays significant roles, was described.

In the end, we want to express deep thanks to Prof. Y. Kinoshita and other members, who gave us a chance to present this lecture. Also deep thanks were devoted to Dr. T. Ino and S. Shibata for their helpful works.