ERYTHROCYTOSIS IN THIAMINE DEFICIENT RATS

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Abstract—A thiamine (T) deficient state in rats was produced by feeding the rats a T deficient diet (TDD). At the stage of 13 days (TDD13 group), the number of red blood cells (RBC) and white blood cells (WBC), hematocrit (Ht) and hemoglobin (Hb) values decreased. On the other hand, after 30 days on the TDD (TDD30 group), the number of RBC was $819 \times 10^4/\text{mm}^3$ as against $631 \times 10^4/\text{mm}^3$ in the normal control group (NC group). Ht and Hb values also increased in the TDD30 group. These changes observed in the TDD30 group were significantly different from findings in the equal weight control group (EWC group) or in the pair fed control group (PFC group). The number of reticulocytes increased, the levels of 2,3-diphosphoglycerate (2,3-DPG) of RBC decreased and plasma erythropoietin levels increased in the TDD30 group. T levels of blood in the TDD13 group were 62 (39–79) ng/ml as against 275 (196–412) ng/ml in the NC group. T levels of blood in the TDD30 group were 102 (17–365) ng/ml, and widely varied. Decrease in 2,3-DPG produces an increase in O_2 affinity to Hb, and hypoxia is induced in the peripheral tissues. Furthermore these conditions stimulate erythropoietin production and finally the number of RBC increases. T deficiency produces anemia at an early stage and absolute erythrocytosis occurs at the late stage of T deficiency. The increased osmotic resistance of RBC in hypotonic solution was also observed in the TDD30 group. This increase in osmotic resistance correlated with the decrease in cholesterol and phospholipid levels in the membrane of RBC.

Thiamine (T) plays an important role on glycolysis (1) which is necessary to perform the normal function and viability of RBC. T-responsive megaloblastic anemia has been reported (2, 3). In an attempt to clarify the relationship hematogenesis, RBC function and T, we carried out various hematological tests on rats maintained on diets deficient in T. Our findings are reported herein.

MATERIALS AND METHODS

1. Animals and diet preparation: Male Sprague-Dawley rats weighing about 60 g were caged individually and fed a regular diet (RD) (Oriental Kobo Co. Ltd. which

contained 0.9 mg T/100 g) for the normal control group (NC group), a T deficient diet (TDD) for the TDD group (TDD group), and TDD plus 2.4 mg T/100 g for the pair fed control group (PFC group). The animals were fed a RD, of which weight was equally adjusted to that of the TDD group, for the equal weight control group (EWC group). The intake of water in the EWC group was also restricted as equal as in the TDD group. Various examinations were done at 13 days and 30 days fed a each diet. Rats were fasted for 18 hr, and blood sample was collected from the vena cava in a heparinized syringe.

2. Hematological examinations and T measurement: RBC and WBC were counted by Thoma's method after adequate dilution. Hematocrit (Ht) value was measured by ultracentrifugal system using capillary. Hemoglobin (Hb) concentration was assayed by Unikit hemoglobin-A (Chugai-Seiyaku Co. Ltd.). Reticulocytes were counted after dyeing by 0.5% brilliant cresyl blue. 2,3-DPG value was assayed by Sigma's 2,3-DPG kit. Plasma erythropoietin levels were assayed by immunological method (Lack Laboratory Co. Ltd.). Plasma was collected about 1 ml from each group for the assay of erythropoietin. T levels of blood and liver were assay by the biological method using *Lactobacillus viridescens* (4). Osmotic resistance of RBC was measured in various NaCl concentrations. One drop of blood was added into NaCl solution of various concentrations and incubated for 1 hr at 37°C, and centrifuged for 10 min at 3,000 rpm, and optical density of the supernatant was measured at 540 nm. The values were expressed as the percent to the optical

density of 100% hemolysis. Lipids of RBC membrane were extracted by Bragdon's method (5). Cholesterol was assayed by Zak's method (6), and phospholipid was assayed by Fiske-Subbarow's method (7).

RESULTS

1. Body weight gain and general symptoms: Body weight gain in the TDD group decreased from about 10 days after feeding a TDD, and at 30 days it was reduced to about 40% of the NC group. Body weight gain in the PFC group was same as that in the NC group (Fig. 1). In the TDD group, some neurological symptoms as abnormal gait were observed at about 25 days, and bleeding in left testis or both testes was found in some rats of this group at 30 days, but these findings were rarely seen in the EWC group. Congestion in mesenteric vein was observed in the TDD30 group.

2. Changes of blood components: The number of RBC decreased to $475 \times 10^4/\text{mm}^3$ in the TDD13 group, and increased to $819 \times 10^4/\text{mm}^3$ in the TDD30 group as

Fig. 1. Body weight gain. NC: normal control group, PFC: pair fed control group, EWC: equal weight control group, TDD: thiamine deficient diet group.

against $631 \times 10^4/\text{mm}^3$ in the NC group. This change was significantly different from the EWC group. (Fig. 2). The number of WBC decreased to about $4,000/\text{mm}^3$ in the

EWC and TDD30 group as against $5,850/\text{mm}^3$ in the NC group (Fig. 3). Ht and Hb values in the TDD30 group were 48.5% and 15.8 g/dl as against 40.5% and 12.5 g/dl in the NC

Fig. 2. Numbers of red blood cells (RBC) in TDD group compared with those in NC group, PFC group and EWC group. TDD 30: rats fed on TDD for 30 days, TDD13: rats fed on TDD for 13 days. Abbreviations are the same as Fig. 1. * $p < 0.001$ corresponding NC group and $p < 0.01$ corresponding EWC group.

Fig. 3. Numbers of white blood cells (WBC) in TDD group compared with those in NC group, PFC group and EWC group. Abbreviations are the same as Fig. 2. * $p < 0.05$ corresponding NC group, ** $p < 0.02$ corresponding NC group.

group, respectively, and these increased significantly rather than those in the EWC group (Fig. 4 and Fig. 5). Mean corpuscular volume (MCV) was $78.5 \mu^3$ in the TDD13 group, and $58.0 \mu^3$ in the TDD30 group as

against $62.0 \mu^3$ in the NC group. Mean corpuscular hemoglobin concentration (MCHC) increased slightly in the TDD and EWC group (Fig. 6). 2,3-DPG, which mediates the binding ability of Hb to oxygen

Fig. 4. Hematocrit values (Ht). Abbreviations are the same as Fig. 2. * $p < 0.001$ corresponding NC group and $p < 0.01$ corresponding EWC group.

Fig. 5. Hemoglobin concentrations (Hb). Abbreviations are the same as Fig. 2. * $p < 0.001$ corresponding NC group and $p < 0.02$ corresponding EWC group.

(O_2), was $3.57 \mu\text{M}/\text{mlRBC}$ in the TDD30 group. 2,3-DPG levels in the TDD30 group was markedly reduced as against $7.93 \mu\text{M}/\text{mlRBC}$ in the NC group (Fig. 7). Reticulocytes were not detected in the EWC

group, and were infrequently detected in the TDD30 group, but some cases in the TDD30 group, the number of them was about 61‰ as against 30‰ in the NC group. Plasma erythropoietin levels were 45 millim-

Fig. 6. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Abbreviations are the same as Fig. 2.

Fig. 7. Levels of 2,3-diphosphoglycerate (2,3-DPG). Abbreviations are the same as Fig. 2. * $p < 0.001$ corresponding NC group and EWC group.

munochemical U/ml in the TDD30 group as against 15 millimunochemical U/ml in the NC group.

3. T levels of blood and liver: T levels of blood decreased markedly to 62 ng/ml in

the TDD13 group, and 102 ng/ml in the TDD 30 group as against 275 ng/ml in the NC group, but T levels at 30 days varied widely from 17 to 365 ng/ml. T levels in the PFC and EWC group were same as that in

Fig. 8. Blood and liver thiamine levels. Abbreviations are the same as Fig. 2. * $p < 0.01$ corresponding NC group and EWC group, ** $p < 0.001$ corresponding NC group and EWC group.

Fig. 9. Percent of NaCl solution, causing 70% hemolysis. Abbreviations are the same as Fig. 2. * $p < 0.001$ corresponding NC group and EWC group.

the NC group. T levels of liver decreased to $0.5 \mu\text{g/g}$ in the TDD30 group as against $15.3 \mu\text{g/g}$ in the NC group, but the levels in the EWC group were equal to that in the NC group. In the PFC group its levels decreased

to $2.4 \mu\text{g/g}$ as against in the NC group (Fig. 8).

4. Properties of RBC membrane: The concentration of NaCl solution that causes 70% hemolysis was 0.41% in the TDD13

Fig. 10. Cholesterol and phospholipid levels of plasma. Abbreviations are the same as Fig. 2. * $p < 0.02$ corresponding NC group.

Fig. 11. Cholesterol and phospholipid levels of RBC. Abbreviations are the same as Fig. 2. * $p < 0.02$ corresponding NC group, ** $p < 0.01$ corresponding NC group and $p < 0.02$ corresponding EWC group.

group and 0.37% in the TDD30 group as against 0.41% in the NC group (Fig. 9). That is, osmotic resistance of RBC to hypotonic solution increased in the TDD30 group, but it was not changed in RBC of the TDD13 and EWC group. Cholesterol and phospholipid levels of plasma increased in the PFC and TDD13 group, but those levels decreased slightly in the TDD30 group (Fig. 10). Cholesterol and phospholipid levels of RBC membrane in the TDD30 group were $124 \mu\text{g}/10^8$ cells and $198 \mu\text{g}/10^8$ cells as against $169 \mu\text{g}/10^8$ cells and $312 \mu\text{g}/10^8$ cells in the NC group, respectively (Fig. 11). Phospholipid levels in the TDD30 group decreased significantly as against the EWC group.

DISCUSSION

Decrease in number of RBC, WBC and Ht values has been reported in T deficient experiment in man (8). We observed the same result in rats fed a TDD for 13 days. As the body weight gain began to decrease after 10 days fed a TDD, so this stage is attributed to early stage of T deficiency and may be correspond to the T deficiency in man. Blood T levels in the TDD13 group were 15 to 20% of that in the NC group. Recently Yokomine et al. reported that the blood T levels in man were 68.1 ± 31.2 ng/ml (9). Murata et al. reported that the blood T levels of boarding girls were 41 ± 3.0 ng/ml, but among them there were many girls whose T levels were very low without any symptoms of beriberi (10). Their T levels were 14 to 24% of the mean T levels. T does not appear to be stored in the body to any appreciable extent; consequently, deficiency symptoms may be observed within a few weeks in subjects maintained on a deficient diet (11). But considering the T levels of rats and humans without any symptoms, there seems to be some T reserve in body to perform normal metabolism. When the blood T levels

decrease to about 20% or less of the mean T levels, symptoms due to T deficiency may be observed. On the other hand, increase in RBC, Ht and Hb values were observed among the animals in the TDD30 group, and these findings were significantly different from the EWC group. Decrease in WBC may result from the chronic malnutrition. The levels of 2,3-DPG in RBC, which plays an important effect on the binding capacity of Hb to O_2 (12), decreased markedly in the TDD30 group, and those were about 30% of that in the NC group. Decrease in 2,3-DPG causes the binding Hb to O_2 tightly, so it is difficult for Hb to release O_2 into peripheral tissues. The hypoxia in peripheral tissues stimulates the production of erythropoietin, so that more RBC are produced. Reticulocytes were not detected in the EWC group and detected infrequently in the TDD30 group, but in some cases in the TDD30 group, the number of them increases about twice of that in the NC group. The blood T levels in the TDD13 group were 62 ng/ml, while the levels in the TDD30 group were 102 ng/ml and very variable from 17 to 365 ng/ml. The transient increase in blood T levels occurring at the late stage of T deficiency is attributed to the mobilization of T in the body to compensate the reduced RBC metabolism. The chronic reduction of glycolysis by T deficiency leads to the diminution of 2,3-DPG levels. Erythrocytosis may be partly due to the decrease in 2,3-DPG levels. As blood T levels in the TDD30 group, which produce erythrocytosis, were higher than that in the TDD13 group which produced anemia, T is an important factor for hematopoiesis. T-responsive megaloblastic anemia has been reported (2, 3). The results that the anemia occurred at early stage of T deficiency and the erythrocytosis at late stage strongly suggested that T plays a key role in hematopoiesis.

T levels of liver were reduced gradually as progression of T deficiency in the TDD

group. But in the PFC group T was reduced to the levels of 2.4 $\mu\text{g/g}$ at 30 days, while at 13 days the levels (16.2 $\mu\text{g/g}$) were the same as those in the NC group. The PFC group did not show any symptoms of T deficiency, so this group was not in T deficient state. At 13 days fed a TDD, the growth rate began to decrease, and the T levels of liver were similar to those in the PFC group at 30 days. Liver needs T to maintain its own metabolism in addition to the role of T storage. So T levels in the PFC group at 30 days are considered to be the minimum concentration to perform its normal metabolic functions. It is not obviously understood why the T levels of liver were low in the PFC group at 30 days.

Comparing the TDD group with the PFC group, lipids levels of plasma were not changed at early stage of T deficiency, but those decreased at late stage of T deficiency. Ando et al. reported that osmotic resistance of RBC is related to the decrease in cholesterol of membrane (13). The similar phenomenon was observed in the TDD30 group, and this increased osmotic resistance to hypotonic solution was the results of the decrease in cholesterol and phospholipid of RBC membrane.

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