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Thrombocytopenia in Graves' Disease: Effect of T_3 on Platelet Kinetics

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Abstract. A study was carried out in which the platelet count was decreased in approximately half the patients with hyperthyroidism and gradually increased with treatment. Platelet disappearance curves were curvilinear and the platelet survival was shortened in the hyperthyroid state. Patients maintained in a euthyroid state for 3 months or less continued to have a shortened platelet survival. The survival returned to normal after 6 months or more of euthyroid status. In order to clarify the cause of the decreased platelet count in the patients, animal experiments were performed. T_3-injected rats had decreased platelet counts and shortened platelet survival. When platelets obtained from T_3-injected rats were transfused to a control group of untreated rats, the platelet survivals were normal. When platelets obtained from the control group of rats were transfused to T_3-injected rats, the platelet survivals were shortened. Disappearance of heat-damaged RBC from the circulation was also accelerated in T_3-injected rats. This suggests that thrombocytopenia in Graves' disease is caused by an increased sequestration potency of the reticuloendothelial phagocyte system stimulated by thyroid hormone.

Thrombocytopenia occurs frequently in Graves' disease [1]. Several reports have been published on the close relationship between the platelet count and the thyroid function in Graves' disease [2, 3], where the platelet count is decreased in the hyperthyroid state and gradually increases as thyroid function returns to normal. *Lamberg et al.* [2] reported a shortened platelet life span in Graves' disease. *Girsh and Myerson* [4] suggested that hypersplenism might be the possible factor of thrombocytopenia in this disease, since splenomegaly is frequently associated with hyperthyroidism. The mechanism of thrombocytopenia in Graves' disease is, however, still a matter of speculation.

In order to analyze the mechanism of the decreased platelet count in Graves' disease, the effect of thyroid hormone on the platelet count and the platelet life span was studied both in patients with Graves' disease and T_3-injected experimental animals.

Materials and Methods

Platelet counts in 214 patients with Graves' disease were examined. The diagnosis of Graves' disease was based on clinical evaluation and the results of a number of laboratory tests including serum T_3, T_4 and cholesterol levels. Untreated patients, patients receiving antithyroid drugs or ^{131}I-radiation but still in a hyperthyroid state, and patients who had returned to a euthyroid state were included in the study. Platelet kinetic studies were performed in 16 patients. These patients were divided into three groups. The first group consisted of 5 hyperthyroid patients, treated and untreated. Mean T_3 and T_4 levels in this group were 414 ± 131 ng/dl (normal 159 ± 20 ng/dl) and 18.9 ± 3.5 μg/dl (normal 8.1 ± 1.3 μg/dl), respectively. The second group consisted of 4 treated patients who were in the euthyroid status for 3 months or less and the third group consisted of 7 patients maintained in the euthyroid status for 6 months or more. Mean T_3 and T_4 levels in these euthyroid patients were 177 ± 12 ng/dl and 9.0 ± 1.5 μg/dl, respectively.

Platelet counting was carried out according to the method of *Brecher and Cronkite* [5]. A platelet kinetic study was performed with autologous platelets, labeled *in vitro* with ^{51}Cr according to the method of *Abrahamsen* [6]. Platelet kinetic studies in rats were done according to the method of *Aster* [7].

The animals used in these experiments were Wistar strain male rats weighing 180–220 g. Each group consisted of 6–10 rats. In order to produce hyperthyroid rats, 20 μg of 3,3'5-triiodo-*L*-thyronine (Nakarai Chemicals, Kyoto, Japan) in physiologic saline adjusted to pH 9.2 by the addition of NaOH was given to each rat intraperitoneally every day. Gain in body weight of T_3-injected rats was 1.4 ± 1.0 g/day and of saline-injected rats was 4.8 ± 1.4 g/day ($p < 0.001$). After T_3 injection was continued for 12 days, serum T_3 level was examined. 1.5 h after the last T_3 injection, T_3 level was 899 ± 182 ng/dl, and 24 h later, T_3 level was 633 ± 145 ng/dl. T_3 level was 76 ± 32 ng/dl in control rats.

The clearance rate of heat-damaged RBC from circulation was measured according to the method of *Kaplan and Jandl* [8]. The labeled RBC was heated for 40 min at 45 °C, thereafter 0.5 ml of ^{51}Cr-labeled RBC suspension was transfused into a rat via a tail vein. 20 μl of the blood sample were obtained from a tail vein at 1, 15 and 24 h following injection. The statistical analyses were made using Student's t test and calculated on differences between matched pairs.

Results

The platelet counts of 214 patients with Graves' disease are shown in figure 1. It was not uncommon for the platelet count to be between 10 and $15 \times 10^4/\mu$l in untreated patients, being of normal value in approximately half the patients. The platelet count in hyperthyroid patients began to increase with treatment with antithyroid drug or ^{131}I-radiation and had returned to a normal level after hyperthyroidism had been well controlled. There was no statistical correlation between platelet count and T_3 or T_4 level.

Platelet kinetic studies in Graves' disease gave the following results. Hyperthyroid pa-

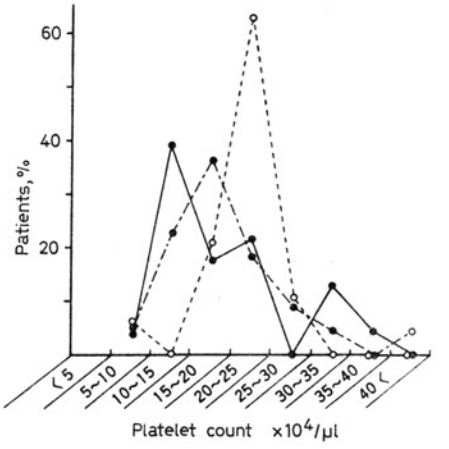

Fig. 1. Platelet count in 214 patients with Graves' disease. ●———● = Before treatment; ●– – – – –● = after treatment, hyperthyroid state; O– – – – – –O = after treatment, euthyroid state.

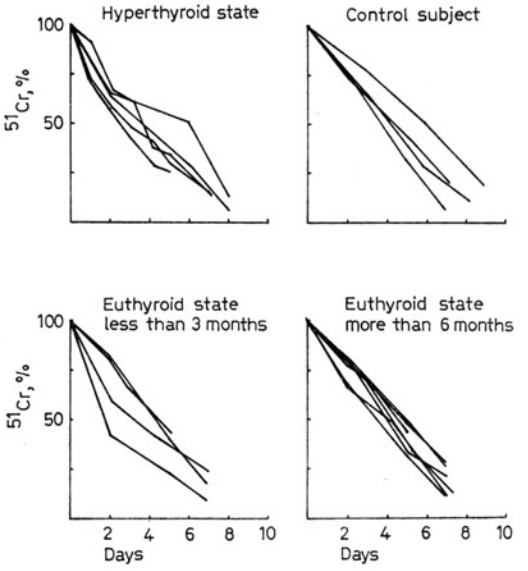

Fig. 2. Platelet survival curves in patients with Graves' disease.

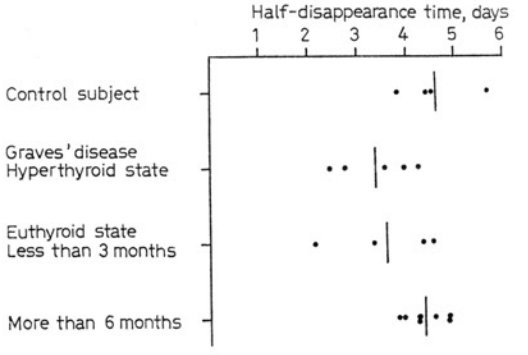

Fig. 3. changes in platelet half-disappearance time in Graves' disease.

tients had curvilinear platelet disappearance curves (fig. 2) and significantly shortened survival of autologous platelet ($T_1/_2 = 3.3 \pm 0.8$ days) as compared to control subjects (4.6 ± 0.8 days) ($p < 0.05$) (fig. 3). There was no significant correlation however, between platelet survival and T_3 or T_4 level. Patients maintained in a euthyroid state for 3 months or less still had a shortened platelet survival (3.5 ± 1.1 days) ($p < 0.05$), similar to hyperthyroid patients. Patients who were euthyroid for 6 months or more had linear platelet survival curves and normal platelet life spans (4.3 ± 0.4 days).

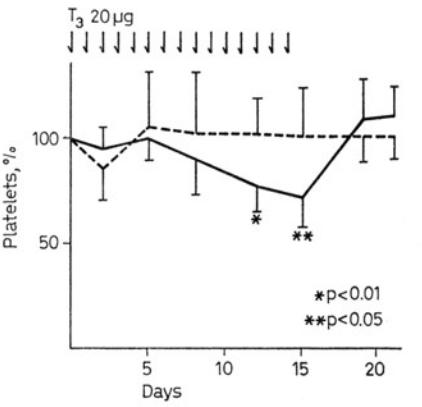

Fig. 4. Time course of changes in platelet count after triiodothyronine injection in rats. - - - - - = Control rat; —— = T_3-injected rat.

Animal experiments were performed to confirm these clinical findings. Hyperthyroid rats were produced by intraperitoneal injection of triiodothyronine. A decrease in the platelet count appeared on the 8th day (fig. 4) and was marked by day 12 ($p < 0.01$) and 15 ($p < 0.005$). The platelet count rapidly returned to normal after the withdrawal of T_3.

Platelet kinetic studies performed on the 8th day of T_3 injection showed a curvilinear platelet disappearance curve and shortened platelet survivals ($T_{1/2} = 28.6 \pm 11.6$ h) as compared with control rats (42.9 ± 2.5 h) (fig. 5). To investigate whether the effect of T_3 on platelet survival resulted from the

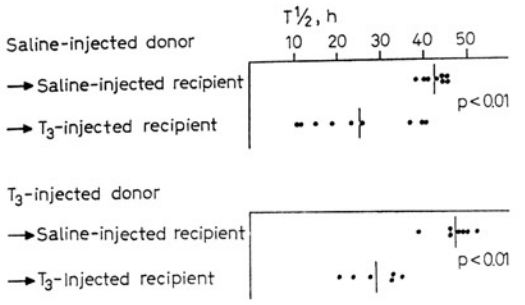

Fig. 5. Platelet half-disappearance time in cross-transfused rats.

change of the platelet itself or other factors, cross-transfusion of platelets was performed. When platelets obtained from T_3-injected rats were transfused to saline-injected rats, platelet survival curves were linear and half-disappearance times were normal (47.1 ± 4.4 h). On the contrary, when platelets obtained from saline-injected rats were transfused to hyperthyroid rats, survival times were shortened (24.8 ± 11.6 h).

When platelets obtained from rats receiving T_3 for 8 days prior to the measurement were transfused to rats receiving T_3 on the day of the survival measurement, platelet survival was normal. Platelet survival on the 3rd day after cessation of 15 days of T_3 injection was measured in order to study when it returned to normal. These rats had slightly shortened platelet survival but the difference was not significant. On the 10th day platelet survival returned to normal.

In order to measure the sequestration activity of the reticuloendothelial phagocyte system (RES), clearance rates of heat-damaged RBC in T_3-injected rats were examined. RBC ^{51}Cr activities (percent injected) in saline-treated rats at 15 and 24 h were 54.6 ± 5.6 and 43.2 ± 8.0, while in T_3-injected rats they were 37.3 ± 7.2 and 24.8 ± 6.2, respectively. The difference was marked at both hours ($p < 0.001$, $p < 0.001$).

Discussion

Thrombocytopenia is a common feature of Graves' disease. *Biström* [1] reported that severe hyperthyroid patients generally had a lower platelet count than moderate hyperthyroid patients. The results of our survey of platelet counts in patients with Graves' disease clearly indicated that a decreased number of platelets was seen in hyperthyroid state. It was also commonly found that the platelet count was decreased in accordance with the degree of hyperthyroid state in ITP patients associated with Graves' disease and began to increase as these patients had normal thyroid function tests [2, 3]. These findings suggest that there is a close relationship between the platelet count and thyroid function.

The mechanism of the shortening of platelet life span is suspected to be as follows:

(1) Autoimmune mechanism – Graves' disease has been considered as an autoimmune disorder since long-acting thyroid stimulators were demonstrated [9]. Circulating immunocomplex was found in these patients and the platelet might be damaged by such a substance. However, platelet antibody or antibody-like substance has not been reported in Graves' disease. In our data, however, patients who were euthyroid for 6 months or more had a normal platelet life span while the etiologic factor of Graves' disease could not be eliminated. According to our unpublished data, patients with Hashimoto thyroiditis, an example of typical autoimmune disease, had normal platelet survivals. These findings suggest that

autoimmunity does not play a main role in the shortening of platelet survival in Graves' disease.

(2) Thyroid hormone – Platelets might be damaged by thyroid hormone in their survival. However, patients maintained in a euthyroid state for 3 months or less had still shortened platelet survivals. Moreover, in cross-transfusion experiments, the platelets obtained from T_3-injected rats had normal survivals in rats given T_3 from the day of transfusion. It was the adequate level in the initial 3 days. These findings suggest that platelets are not directly damaged with thyroid hormone.

(3) RES system – Spleen and/or reticuloendothelial phagocyte system may be activated by thyroid hormone. Hyperplasia of these organs is commonly found in Graves' disease [10]. The spleen was considered as a main sequestration site of platelets [11]. In our data, the platelet count gradually was decreased with T_3 injection and the significant shortening of platelet survival was observed after 8 days of injection. We considered that the reticuloendothelial phagocyte system in rats might be activated with T_3. Various methods have been reported to measure phagocytic activity of reticuloendothelial organs. Accelerated disappearance of heat-damaged RBC from the circulation was revealed in hyperthyroid rats. *Frimmer* [12] also has reported that reticuloendothelial clearance for macromolecules was accelerated in hyperthyroid rats.

Patients maintained in a euthyroid state for 3 months or less still had a shortened platelet survival in our study. This indicates that clearance rate of phagocytic organs does not concomitantly return to normal with T_3 level. The rate gradually returns to normal after serum T_3 level is normalized. Therefore there was no correlation between platelet count or platelet survival and T_3 or T_4 level.

We consider that the mechanism of the shortened platelet survival resulting in thrombocytopenia in Graves' disease is an increased sequestration activity by the reticuloendothelial phagocyte system stimulated with thyroid hormone.

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