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Platelet Epidermal Growth Factor in Thyroid Disorders

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Abstract. We evaluated the concentration of epidermal growth factor (EGF) in platelets, serum and plasma obtained from 47 patients with Graves' disease, 7 with hypothyroidism and 20 healthy subjects. The platelets of the subjects were collected from platelet rich plasma and lysed by freezing and thawing. Subsequently the platelet debris was treated with Triton X-100. The EGF concentration was determined by homologous radioimmunoassay. The concentration of EGF in the platelets in 14 patients with untreated Graves' disease was significantly higher than that in the healthy controls. After treating these 14 patients with antithyroid agents, the EGF concentration in the platelets decreased to the level of the healthy controls. The EGF concentration in the platelets in the 7 untreated hypothyroid patients decreased after replacement therapy with thyroxine. The mean volume of the platelets in the 14 patients with untreated Graves' disease was significantly larger than in the control and decreased after treatment with antithyroid agents. The serum and plasma levels of EGF in the 7 untreated hypothyroid increased after replacement therapy. In conclusion, thyroid function affected the concentration of EGF in the platelets of patients with thyroid disorders.

Key words: Epidermal growth factor (EGF), Thyroid hormone, Platelet, Graves' disease, Hypothyroidism
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THYROID hormones are known to alter the content of epidermal growth factor (EGF) in several tissues such as skin, kidneys, submaxillary gland, ocular tissue and thyroid glands [1–8]. Large amounts of EGF also exist in human platelets [9, 10]. We previously reported increased EGF content in platelet lysates from diabetics [11]. EGF content in platelets may vary with certain pathologic conditions, but it is not known whether there is any alteration in the EGF content of platelets from patients with thyroid disorders. EGF has a potent *in vitro* inhibitory effect on iodine uptake to thyroid follicle cells in pigs, dogs, sheep, and man [13]. EGF also inhibits thyroidal secretion of T3 and T4 *in vitro* and *in vivo* [13–16], but the physiological role of EGF in human platelets remains unclear. The EGF released from activated platelets in thyroid tissues may act in a paracrine fashion, resulting in the above-mentioned functional changes in the thyroid glands. The association between the circulating EGF in plasma or platelet-derived EGF and thyroid function has not been fully investigated. The purpose of this study was to determine whether the thyroid function affected the concentration of EGF in platelets, plasma and serum of patients with thyroid dysfunction.

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Subjects and Methods

Subjects

We studied 54 Japanese outpatients, 47 with Graves' disease and 7 with hypothyroidism, and 20 healthy subjects. The patients with Graves'

disease were diagnosed on the basis of their laboratory data, physical manifestations and iodine uptake. All the patients with Graves' disease were treated with thiamazole and propylthiouracil. Fourteen of the patients with Graves' disease and all those with hypothyroidism were studied before the treatment and also after it when they became euthyroid. As a control group, we examined 20 clinically healthy subjects who were not taking any drugs that are known to affect platelet function. The levels of serum T3, T4, TSH and platelet counts, mean platelet volume (MPV), platelet crit (PCT), serum or plasma EGF in the untreated and treated patients with Graves' disease, in those with hypothyroidism and in the healthy controls are shown in Table 1.

Sample preparation

To increase the recovery rate of EGF from the platelets by our previous methods [10], we prepared the samples as described below. Platelet-rich plasma (PRP) was used to measure the MPV and PCT with a Coulter hematology analyzer (Coulter, Hialeah, Fla, USA). Next, 250 μl of PRP was immediately centrifuged at 3000 × g for 60 min. The supernatants were used as plasma samples. The platelet pellets were resuspended in a PCT dependent volume of 50 mM Tris-HCl buffer to obtain the platelet lysates with the same volume of platelets. After six cycles of freezing and thawing, the samples were centrifuged at 3000 × g for 60 min. Finally, the platelet debris was sonicated in a 0.1% Triton X-100 solution and mixed with the supernatant [11].

In a pilot study, we observed that the EGF in the platelets released into serum and the serum EGF level reached a plateau at 3 h at room temperature. To obtain serum samples, the test tubes were allowed to stand at room temperature for 5 h and then centrifuged at 1800 × g for 20 min.

EGF measurement

A double antibody radioimmunoassay for human EGF was performed as described Gregory [17]. Recombinant human EGF (Earth Chemical Co., Hyogo, Japan) was radioiodinated by the chloramine T method. Antibody-bound labeled ligand was separated from unbound ligand by a double-antibody procedure. The inter- and intra-assay coefficients of variation were 3.5% and 6.2%, respectively.

Evaluation of remaining EGF in platelet debris

To compare the recovery of immunoreactive epidermal growth factor (irEGF) employing freezing and thawing alone [11] with the protocol used in this study, we examined the residual EGF concentration in the platelet debris from 7 patients with untreated Graves' disease, 7 patients with untreated hypothyroidism and 7 healthy subjects. The platelet lysates after freezing and thawing were

Table 1. Subjects' characteristics and data for platelet and EGF in serum, plasma and platelets

	Hyperthyroid		Hypothyroid		Healthy controls
	Before treatment	After treatment	Before treatment	After treatment	
N (men: women)	14 (3:11)		7 (1:6)		20 (5:15)
Age (year)	34 ± 10		42 ± 19		44 ± 22
Serum T3 (ng/dl)	388 ± 133	179 ± 48**	77 ± 28	108 ± 48**	
Serum T4 (μg/dl)	17.2 ± 5.5	8.4 ± 3.9**	3.3 ± 1.5	6.7 ± 4.5**	
Serum TSH (μU/ml)	0.07 ↓	5.4 ± 0.3**	174 ± 108	20.5 ± 18.1**	
Platelet counts ($\times 10^4/\mu l$)	26.5 ± 9.1	27.4 ± 8.8	26.4 ± 8.2	25.9 ± 10.1	27.4 ± 7.1
Mean platelet volume (fl)	6.80 ± 0.69	6.45 ± 0.60**	6.52 ± 0.68	6.60 ± 0.45	6.52 ± 0.34
Platelet crit (%)	0.180 ± 0.036	0.176 ± 0.039	0.172 ± 0.039	0.171 ± 0.040	0.178 ± 0.031
Serum EGF (ng/ml)	2.2 ± 0.9	2.5 ± 1.3	1.5 ± 0.8	2.6 ± 1.6*	2.4 ± 0.8
Plasma EGF (pg/ml)	115 ± 51	148 ± 42	54 ± 18	128 ± 82*	134 ± 41
Platelet EGF (ng/mm^3 plt)	0.449 ± 0.185	0.342 ± 0.078**	0.491 ± 0.221	0.363 ± 0.178**	0.329 ± 0.104

Values are the mean ± SD. Significant differences between before and after treatment (paired *t*-test) *, $P<0.05$, **, $P<0.01$. EGF, epidermal growth factor.

centrifuged, and then the supernatants or pellets were treated as described below. We determined the content of EGF in the supernatants alone and in the mixed samples consisting of the supernatant and the lysed debris, as described in the sample preparation. We then calculated the percentage of EGF in the platelet debris relative to that in the mixed sample.

Statistical analysis

Changes due to treatment in thyroid patients were assessed by paired *t*-test. For all variables, differences between the groups were assessed by Student's *t*-test. A simple linear regression was used in analysis of the correlation between the platelet epidermal growth factor (PL-EGF) and thyroid hormone. Pearson's correlation coefficient (r) was determined to assess the significance of correlation. A *P* value less than 0.05 was considered statistically significant.

Results

EGF values for serum, plasma and platelets

The serum or plasma level of EGF was approximately the same before and after treatment in Graves' disease patients. These levels in patients with untreated Graves' disease did not differ from those in the healthy controls. The serum EGF levels in the patients with untreated hypothyroidism were much lower than after treatment with L-thyroxine replacement therapy. The plasma EGF concentrations in patients with untreated hypothyroidism were also much lower than those in patients after treatment and in the healthy controls (Table 1).

We diluted the platelet sample according to the PCT to determine more accurately the content of the irEGF in the same volume of platelets (expressed as ng per mm^3 platelet volume; ng/mm^3 plt). The PL-EGF concentration in patients with untreated Graves' disease was much higher than in the healthy control (0.449 ± 0.185 *vs.* 0.329 ± 0.104 ng/mm^3 plt: $P<0.01$). The PL-EGF of the 14 patients with untreated Graves' disease decreased appreciably after treatment with antithyroid agents (from 0.449 ± 0.185 to 0.342 ± 0.078 ng/mm^3 plt: $P<0.05$). In three of the 14 patients, the PL-EGF after treatment was higher than before it. The TSH level after treatment of all three patients was greater than 10 μU/m*l*. The PL-EGF in the 7 untreated hypothyroid patients was much higher than in the healthy controls (0.491 ± 0.221 *vs.* 0.329 ± 0.104 ng/mm^3 plt: $P<0.05$) and decreased significantly after replacement therapy with L-thyroxine (from 0.491 ± 0.221 to 0.363 ± 0.178 ng/mm^3 plt: $P<0.01$). There was not a close correlation between the PL-EGF and the patient's age. Furthermore, no sex differences in the PL-EGF in any of the groups, including the patients with Graves' disease and hypothyroidism, and the healthy controls, were observed.

Platelet count, platelet crit and mean platelet volume

The platelet count and MPV and PCT for each patient are shown in Table 1. There were no significant differences in the platelet count or PCT among the 5 groups studied: the treated and untreated Graves' disease patients, the treated and untreated hypothyroid patients and the healthy controls. The MPV of the platelets from the untreated hyperthyroid patients was significantly greater than in the healthy controls and became smaller after treatment with antithyroid agents (Table 1). No significant difference was found between the size of platelets in the untreated and treated hypothyroid patients.

Relation between platelet EGF and serum T4 level

There was no significant correlation between the PL-EGF and the level of serum T4 in the 47 patients with Graves' disease including 11 patients who became hypothyroid after treatment (Fig. 1a). The PL-EGF of untreated hypothyroid patients decreased after replacement therapy with L-thyroxine, so we omitted the data on the 11 patients whose TSH was greater than 10 μU/m*l* (Fig. 1b). Fig. 1b shows that the PL-EGF in Graves' disease patients without hypothyroidism significantly correlated with the serum T4 level ($r=0.48$, $P<0.01$). There was no correlation between the PL-EGF and the serum T3 level. There was a significant correlation between the PL-EGF and serum TSH in the 11 patients with post-treatment hypothyroidism ($r=0.52$, $P<0.05$).

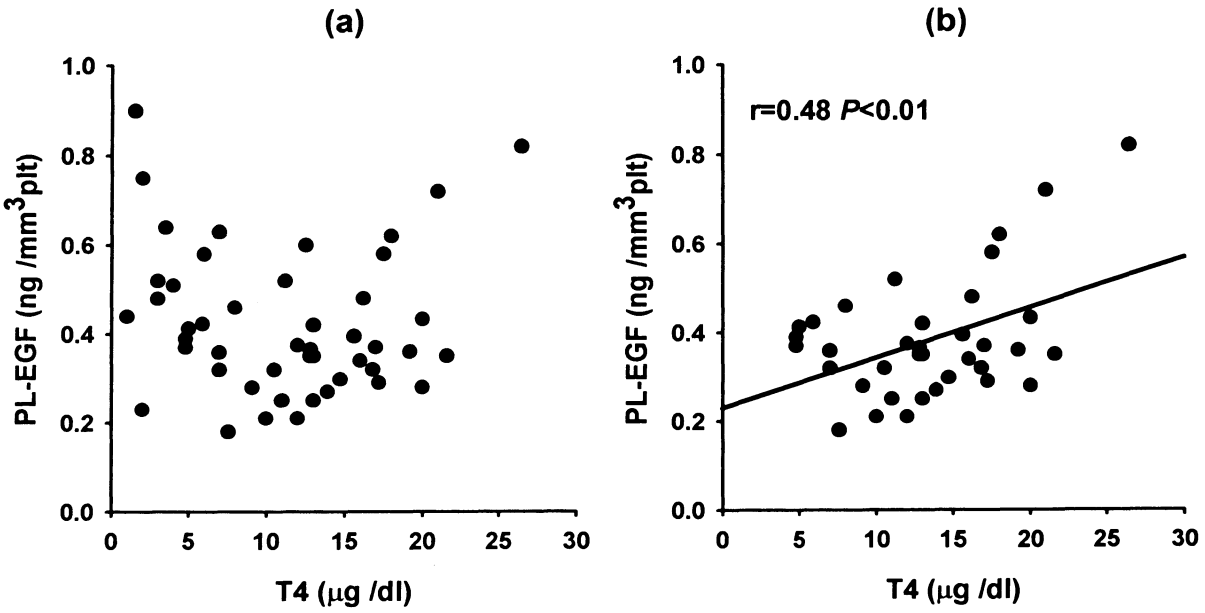

Fig. 1. Relationship between the PL-EGF concentration and the serum T4 level in 47 patients with Graves' disease (Fig. 1a). Relationship between the two parameters shown in Fig. 1a excluding those with Graves' disease who developed hypothyroidism following treatment (Fig. 1b).

EGF in platelet debris

In the samples obtained from the 7 patients with untreated Graves' disease and 7 healthy subjects, the irEGF content of the platelet debris was consistently 30–35% of the total EGF in the platelet lysate. In the samples obtained from the 7 hypothyroid patients, the residual EGF in the platelet debris was 42–68% of the total. The irEGF in the platelet debris of patients with untreated hypothyroidism was significantly higher than in patients with untreated Graves' disease or in the healthy controls.

Discussion

In the present study, PL-EGF was increased in patients with untreated Graves' disease compared with the healthy control. After treatment for hyperthyroidism, the EGF concentration in platelets significantly decreased.

Kung *et al.* [16] reported that serum EGF was increased in hyperthyroid patients compared with the healthy controls. They hypothesized that this elevation is due to increased concentrations of EGF in the platelets obtained from hyperthyroid patients, because serum EGF is derived mainly from platelets [10]. Our findings strongly support their hypothesis. Contrary to the reports by Kung *et al.*, however, we did not find any significant difference between the serum concentrations of EGF in those with hyperthyroidism and the healthy controls, possibly because the standard PCT deviation in our study was larger than in theirs, making small differences difficult to detect.

A possible mechanism for the increased EGF content in platelets is as follows. Because the proteins including EGF in the α-granules of platelets appear to be released gradually and continuously into the circulation and their concentrations can then decrease without aggregation [17, 18], a younger platelet may contain more EGF than an older one. Shortened platelet survival has been observed in many studies of patients with hyperthyroidism, and is thought to be due to enhanced splenic sequestration [19–21]. Accordingly, patients with hyperthyroidism would have greater numbers of younger platelets which contain more EGF. In support of this, it is known that the size of platelets decreases with their age, and the MPV in the patients with hyperthyroidism is larger than in euthyroid patients or normal

subjects [19]. Another possible explanation is that the synthesis of EGF in megakaryocytes may be increased in patients with hyperthyroidism. The EGF in platelets is thought to be synthesized in the megakaryocytes in the bone marrow [22], so that future studies should examine whether thyroid hormone affects the production of EGF in human megakaryocytes.

Contrary to the findings of Kung *et al.* [16], we found that the plasma concentration of EGF in untreated hypothyroid patients was much lower than in the healthy controls and increased after replacement therapy. Qualitative changes in platelets in hypothyroidism were reported [23–26]. Increased remaining EGF in platelet debris in untreated hypothyroid patients may be due to the altered stability of the platelet skeleton or membrane in hypothyroid patients. We suspect that platelets change so as to resist the release of EGF into the circulation, resulting in a decrease in the plasma EGF concentration and consequently an increase in PL-EGF in untreated hypothyroid patients. EGF inhibits the production of thyroid hormones, and, in contrast, and stimulates the growth of thyroid cells [27]. So that the physiological role of the change in PL-EGF in thyroid disorders is not clear and deserves further study.

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