Inhibitory Effect of Thyroid Blocking Antibody (TBAb) on the Thyroid Stimulatory Effect of Human Chorionic Gonadotropin (HCG) and Equine Chorionic Gonadotropin (ECG)

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Abstract. We examined the inhibitory effect of thyroid blocking antibody (TBAb) on the thyroid stimulating activity of human chorionic gonadotropin (HCG) and equine CG (ECG). Five TBAb positive sera obtained from patients who had been hypothyroid but were currently on T4 treatment. The TSH binding inhibitory immunoglobulin (TBI) activities of the sera were 60-160 IU/L. Inhibition of TSH binding to the TSH receptor (TSHR) [TSH binding inhibition (TBI) activity] of HCG or ECG, and inhibition of TBAb on HCG or ECG-stimulated cAMP production was examined. Both HCG and ECG preparations showed weak TBI activity in the presence of small amounts of protein [bovine serum albumin (BSA)] but were negative in the presence of large amounts of protein [normal human serum (NHS) or BSA]. Four thousand IU/mL of HCG and ECG preparation caused cAMP production similar to 100μU/mL of bovine (b) TSH. The inhibitory effect of TBAb on cAMP production by this amount of HCG or ECG was then examined. The inhibitory effect of TBAb on cAMP production by TBAb positive sera with more than 40 iu/L TBii activity completely blocked cAMP production by HCG, ECG and bTSH. This suggests that common alpha-subunit of both HCG and TSH are involved in the inhibitory effect of TBAb. Previous reports demonstrated that the thyroid stimulating activity of thyroid stimulating antibody (TSAb) was blocked by deglycosylated HCG (competitive antagonist of TSH binding to TSHR). The fact and our present study suggest that TSAb, HCG, ECG, and TBAb have a similar binding site (alpha-subunit-mimicking binding site) on the TSH receptor.

Key words: Human chorionic gonadotropin, Equine chorionic gonadotropin, Thyroid blocking antibody, TSH receptor antibody, cAMP

Two kinds of TSH receptor (R) antibody (TRAb) are known. One is the thyroid stimulating antibody (TSAb) active in hyperthyroidism and other is the thyroid blocking antibody (TBAb) causing primary hypothyroidism. TBAb blocks the thyroid stimulating activity of TSH by the inhibition of TSH binding to the TSHR. TBAb also blocks the thyroid stimulating activity of TSAb by inhibition of TSAb binding to the TSHR [1, 2].

Human chorionic gonadotropin (HCG) has the thyroid stimulating activity through binding to the TSHR [3-11]. Deglycosylated HCG (asialo-or asialoagalacto- HCG) is a competitive antagonist of TSH because of increased TSHR binding compared to HCG. An inhibitory effect of asialo-HCG or asialoagalacto-HCG on the thyroid stimulating activity of TSAb has been reported [6-10]. These facts suggest that TSH, TSAb, HCG and deglycosylated-HCG may bind to a similar region of TSH R. In the present study we examined the effects of TBAb on the thyroid stimulating activity of HCG and equine chorionic gonadotropin (ECG).

Materials and Methods

HCG preparation (gonatropin purified from pregnancy urine, 4,271 IU/mg) and ECG preparation (se-
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Volume 0.25mL containing 0.1% bovine serum albumin (BSA). cAMP production by HCG and ECG was increased in a dose-dependent manner. cAMP production by 4,000 IU/mL of HCG (Asuka), HCG (Aspen) and ECG (Asuka) was approximately similar to the effect of 100μU/mL of bTSH (Fig. 1A & B). HCG and ECG-stimulated cAMP production was not stimulated by 5% polyethylene glycol (PEG) (data not shown).

3) TSH binding inhibition (TBI) activity of HCG and ECG

Both HCG and ECG preparations showed positive TBI activities in the presence of small amounts of protein (0.3 mg) of BSA. TBI activities in 4,000 IU/mL of HCG (Asuka), HCG (Aspen) and ECG showed 4.3, 2.0 and 3.0 IU/L, respectively. However, TBI activity of these preparations was negative in the presence of large amounts of protein (8 mg) of normal human serum (NHS) (Fig. 2A & B) or large amounts of BSA (8mg) of BSA (data not shown).

Table 1  TBI/I activity, TSAb activity and TSH level in TBAb-positive hypothyroidism

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Before T4 treatment</th>
<th>During T4 treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TBI* (%)</td>
<td>TBA (%)</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>100</td>
</tr>
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<td>3</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>87</td>
<td>100</td>
</tr>
</tbody>
</table>

Normal range =<10 <40 <180 0.4-4.2

*; TBI/I was determined by TRAb assay kit (1st generation method, RSR Ltd, Cardiff, UK). TSAb and TBA/I was determined by the stimulation of cAMP production by Yamasa kit (Chosi, Ciba, Japan) as previously reported [12-14]. TBA/I activity was determined as 100% when TBA/I activity determined by several assays was distributed to 95-105%.

Results

1) TBI/I activity, TSAb activity and TSH in TBA/I positive patients

Sera were obtained from five hypothyroid patients with high TBI/I activity (>87% TBI/I), positive TBA/I activity and negative TSAb activity, who were treated with T4 for many years. High TBI/I activity of these patients continued in spite of normalization of TSH levels by T4 treatment (Table 1).

2) Thyroid stimulating activity of HCG and ECG

HCG or ECG preparation was incubated in total volume 0.25mL containing 0.1% bovine serum albumin (BSA). cAMP production by HCG and ECG was increased in a dose-dependent manner. cAMP production by 4,000 IU/mL of HCG (Asuka), HCG (Aspen) and ECG (Asuka) was approximately similar to the effect of 100μU/mL of bTSH (Fig. 1A & B). HCG and ECG-stimulated cAMP production was not stimulated by 5% polyethylene glycol (PEG) (data not shown).

Fig. 1. cAMP production by HCG and ECG preparation. Values are mean of duplicate determinations.

3) TSH binding inhibition (TBI) activity of HCG and ECG

Both HCG and ECG preparations showed positive TBI activities in the presence of small amounts of protein (0.3 mg) of BSA. TBI activities in 4,000 IU/mL of HCG (Asuka), HCG (Aspen) and ECG showed 4.3, 2.0 and 3.0 IU/L, respectively. However, TBI activity of these preparations was negative in the presence of large amounts of protein (8 mg) of normal human serum (NHS) (Fig. 2A & B) or large amounts of BSA (8mg) of BSA (data not shown).
EFFECT OF TBAb ON HCG

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Fig. 2. TBI activity of HCG and ECG. HCG and ECG were incubated with BSA (0.3 mg) or NHS (8 mg). Values are mean of duplicate determinations.

Fig. 3. Inhibitory effect of TBAb on the thyroid stimulating activity of HCG (Aspen). BSA (0.3 mg), NHS (PEG 22.5% precipitated fraction of 0.2 mL NHS) and TBAb (No.1) (PEG precipitated fraction with 10-60 IU/L TBII) were used. Values are mean ± SD of triplicate determinations.

Fig. 4. Inhibitory effect of TBAb on the thyroid stimulating activity of HCG (Asuka) and ECG. Values are mean of duplicate determinations.

4) Inhibitory effect of TBAb on the thyroid stimulating activity of HCG

cAMP production of HCG (Aspen) was similarly positive in the presence of BSA (0.3 mg) or NHS (22.5% PEG precipitated fraction of 0.2 mL). The inhibitory effect of TBAb positive serum (No.1) on the thyroid stimulating activity of HCG (4,000 IU/mL, Aspen) was observed by co-incubation of HCG and 22.5% PEG precipitated fraction of TBAb positive serum. The inhibitory effect of TBAb was seen in a dose-dependent manner and >40 IU/L TBII (patient No.1) caused complete inhibition (Fig. 3).

The inhibitory effect of TBAb (5 patients) on cAMP production of HCG (4,000 IU/mL, Asuka) was examined. When 3 TBAb positive sera with high TBII activities (>130 IU/L) (No1-3) were used, cAMP production by HCG was inhibited in a dose-dependent manner, and complete inhibition was observed by >40 IU/L. Two TBAb positive sera (No. 4 and 5) with low TBII activity showed weak inhibitory activities at 20 IU/L (Fig. 4A).
5) Inhibitory effect of TBAb on the thyroid stimulating activity of ECG
These TBAb positive sera showed inhibitory effect on cAMP production by ECG (4,000 IU/mL) similar to that seen using HCG (Fig. 4B).

6) Inhibitory effect of TBAb on the thyroid stimulating activity of bTSH
Inhibitory effect of these TBAb positive sera on bTSH (100 μU/mL) -stimulated cAMP production was also similar to that found with both HCG and ECG (Fig 5).

Discussion
The sera of hypothyroid patients with high TBI activity typically have positive TBAb activities, determined by the inhibitory effect of TBI positive serum on bTSH-stimulated cAMP production in vitro assay using thyroid cells [1, 2, 13]. TSAb positive serum may also be used instead of bTSH for the determination of TBAb activity. Thus, TBAb is the inhibitor of TSHR binding of TSAb or TSH. There are many reports that HCG has the thyroid hormone releasing activity via TSHR binding [3-11]. Furthermore, the thyroid stimulating activity of HCG is blocked by TBAb [11]. Deglycosylated- HCG (asialo- or asialoagalacto-HCG) is a competitive antagonist of TSH binding to TSHR. Because of increased TSHR binding compared to HCG, it has blocking action on cAMP production by TSH and TSAb [6-11]. In the present experiment we examined the inhibitory effect of TBAb on the thyroid stimulating activity of HCG and ECG. More than 70 % and 50 % amino acids in the α- and β-subunits of HCG and ECG are similar [15]. Both HCG and ECG preparations showed weakly positive TBI activities in the presence of small amounts of protein (0.3 mg of BSA) but negative TBI activities in the presence of large amounts of protein (8 mg of NHS or BSA). The binding affinity of HCG or ECG to TSHR is so low that its TSHR binding may be inhibited by large amounts of protein, but not by small amounts of protein. However, both preparations showed similarly positive thyroid stimulating activities in both the presence of small amounts and large amounts of protein. A similar phenomenon was also found in pituitary adenylate cyclase activating polypeptide (PACAP) as described previously [16].

TBAb activity is determined by inhibitory effect of TBAb positive serum on 100μU/mL bTSH-stimulated cAMP production using porcine thyroid cells (Yamasa’s TSAb kit)[12-14]. Four thousand IU/mL of HCG and ECG caused cAMP production similar to 100μU/mL bTSH. Thus, the inhibitory effect of TBAb on HCG and ECG was examined using this amount of CG preparation. Three TBAb positive sera with high TBI activities showed complete inhibition of thyroid stimulating activities of HCG and ECG when using more than 40 IU/L TBI, although 2 patients with low TBI activities showed weak inhibition at 20 IU/L. The inhibitory effect of these tested TBAb positive sera on the cAMP-producing activity of HCG, ECG and bTSH was similar. The inhibitory effect of the same amount of TBI (20IU/L) in these TBI-positive sera on cAMP production by HCG, ECG and bTSH increased in paralleled with TBI activity of the test serum. The fact suggests that TBAb activity in test serum with the same TBI activity may be slightly different. One reason might be the difference of small amounts of TSAb in TBAb positive serum.

The present experiment shows that the α-subunit of TSH, HCG and ECG molecule may participate in the action of these hormones and may influence TSHR binding of TBAb. Studies using monoclonal TRAb indicate that TSH, TSAb and TBAb bind to the similar region of TSHR [17]. Recently, it was found that the heavy and light chain of human thyroid stimulating monoclonal antibody (M22)-Fab mimics TSHα-chain and β-chain in the TSHR binding, respectively [18]. Our study also suggests that TSH and TBAb may have α-subunit-mimicking TSHR binding site. However,
it is still unclear whether TSH and these two kinds of TRAb bind to different portions of the TSHR. Subtle differences in binding characteristics are suggested for the opposite action between TSAb and TBAb. Further studies are necessary to clear the structure-function relationship of TSAb, TBAb, HCG and TSH.

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References