

## Anatabine supplementation decreases thyroglobulin antibodies in patients with chronic lymphocytic autoimmune (Hashimoto's) thyroiditis: A randomized controlled clinical trial

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**Context:** Hashimoto's thyroiditis is less prevalent in tobacco smokers. Anatabine, an alkaloid found in *Solanaceae* plants including tobacco, has been reported to ameliorate a mouse model of Hashimoto's thyroiditis.

**Objective:** The effects of anatabine in patients with Hashimoto's thyroiditis.

**Design, Setting, Patients, and Intervention:** Double-blind, randomized, placebo-controlled multi-site study. A total of 146 patients (70 treated with anatabine, and 76 placebo) completed the study. Approximately 50% of patients in each group were on levothyroxine medication. Anatabine lozenges (9–24 mg/day) or placebo, each containing vitamins A and D3, were administered orally 3 times a day for three months.

**Main Outcome Measures:** Assessment of serum thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb). Safety was assessed through adverse events (AEs), clinical laboratory evaluations, and vital sign measurements.

**Results:** Anatabine treated patients had a significant reduction in absolute serum TgAb levels from baseline by study end relative to those on placebo ( $p=0.027$ ); however, there were no significant changes or differences in treatment group means for TPOAb or TgAb. Mean ( $\pm$ SD) TgAb values decreased by 46.2 ( $\pm$ 101.1) and 3.9 ( $\pm$ 83.9) WHO units for the anatabine and placebo groups, respectively. Significantly more patients had a  $>20\%$  drop in TgAb in the anatabine than placebo group ( $p=0.023$ ). Overall the anatabine supplement was safe and well tolerated, although significantly ( $p<0.05$ ) more patients in the anatabine group reported AEs.

**Conclusions:** These results demonstrate an immunological effect of anatabine on TgAb levels. Further studies are warranted to dissect longer-term effects and possible actions of anatabine on the course of Hashimoto's thyroiditis.

**T**reatment for chronic lymphocytic (Hashimoto's) thyroiditis consists of L-thyroxine replacement when hypothyroidism develops (1). Tobacco smoking has numerous effects on thyroid volume, function, and disease, and a protective effect on development of Hashimoto's thyroiditis and thyroid antibodies (2). Nicotine has anti-inflammatory effects (3) but cannot be recommended because it is addictive (4) and toxic (5, 6). Anatabine, another *Solanaceae* alkaloid with a similar chemical structure, may have immunomodulatory properties. In a mouse model of thyroiditis, anatabine reduces the incidence and severity of thyroiditis and lowers levels of thyroglobulin antibodies (TgAb) (7). We designed a clinical trial to assess effects of anatabine dietary supplementation in patients with Hashimoto's thyroiditis.

## Materials and Methods

### Study sites, patients, and objectives

This was a multicenter, double-blind, placebo-controlled, randomized clinical trial (RCT) enrolling patients with Hashimoto's thyroiditis. IRB approval was obtained and all study patients provided signed informed consent. Patients were recruited from 9 endocrinology clinics in the United States between March 2012 and August 2012.

The primary objective was to collect information on the effects of anatabine supplementation in Hashimoto's thyroiditis patients. Patients taking L-thyroxine were included, but only if their dose was  $\leq 1.0$  mcg/kg/d to exclude individuals with thyroid destruction incapable of responding to any intervention. The main inclusion and exclusion criteria are provided in Supplemental Table 1.

### Study design and randomization

Patients underwent five study site visits over four months. At visit 1 (screening), demographics, vital signs, medical and medication history, and blood and urine samples were collected, and ultrasonography was scheduled. At visit 2 (randomization), patients were randomly assigned to either the anatabine or placebo group. Thereafter, patients returned monthly for visits 3, 4, and 5 to complete study procedures.

### Anatabine and placebo lozenge

Anatabine was provided by Rock Creek Pharmaceuticals (Gloucester, MA), and formulated into a flavored mannitol granulation lozenge that also contained fractional replacement doses of vitamins A (834 IU) and D3 (66 IU), in both active and placebo units to reduce the chance that vitamin deficiencies might obscure an anatabine effect on autoimmunity. Anatabine lozenges were administered orally 3 times daily to a target total dose of 0.17–0.25 mg/kg/d. To reduce nicotinic type effects (eg, dizziness, nausea), patients started with 9 mg/d and advanced to the

target dose during week two. Patients who took less than 70% of assigned treatment (pill count) were excluded from efficacy analysis.

### Study outcomes and assays

The main experimental outcomes were serum TgAb and thyroperoxidase antibodies (TPOAb). Other measures included serum thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), and inflammatory biomarkers (high-sensitivity C-reactive protein (CRP) [hsCRP], interleukin-1 beta [IL-1 beta], IL-6, and IL-18); and ultrasonographic thyroid volume, echogenicity, and vascularity. North Coast Clinical Laboratory (Sandusky, OH) performed the measurements of thyroid function and hsCRP. Assays for TgAb and TPOAb and the three interleukins were performed at Johns Hopkins Immunological Disorder Laboratory (Baltimore, MD). Thyroid ultrasonography was performed at the 9 sites and then sent to a central radiologist who read them blinded (Supplemental Table 2).

### Statistical analysis

The dataset included thyroid-related variables (TgAb, TPOAb, TSH, FT4, FT3, volume, vascularity, and echogenicity), demographic variables (sex, age, race, and ethnicity), body mass index (BMI), inflammatory markers (hsCRP, IL-1 beta, IL-6, and IL-18), and safety outcomes. Urinary iodine was not measured.

We compared nonadjusted continuous variables between the two treatment groups using a paired *t* test when normally distributed, Wilcoxon rank-sum test when not normally distributed, and  $\chi^2$  test for categorical variables.

All statistical analyses were performed using Stata 12 (Stata Corp., College Station, TX) or JMP 7 (SAS Institute, Cary, NC).

## Results

### Demographics and baseline characteristics

Among 230 patients screened, 165 were randomized in the study and 146 completed efficacy evaluation (70 receiving anatabine, and 76 placebo; Supplemental Figure 1). Patients were predominantly female, Caucasian, and non-Hispanic (Supplemental Table 2). Seventy-seven patients reported taking levothyroxine (36 on anatabine and 41 on placebo). There were no significant differences between groups with respect to demographic and baseline characteristics.

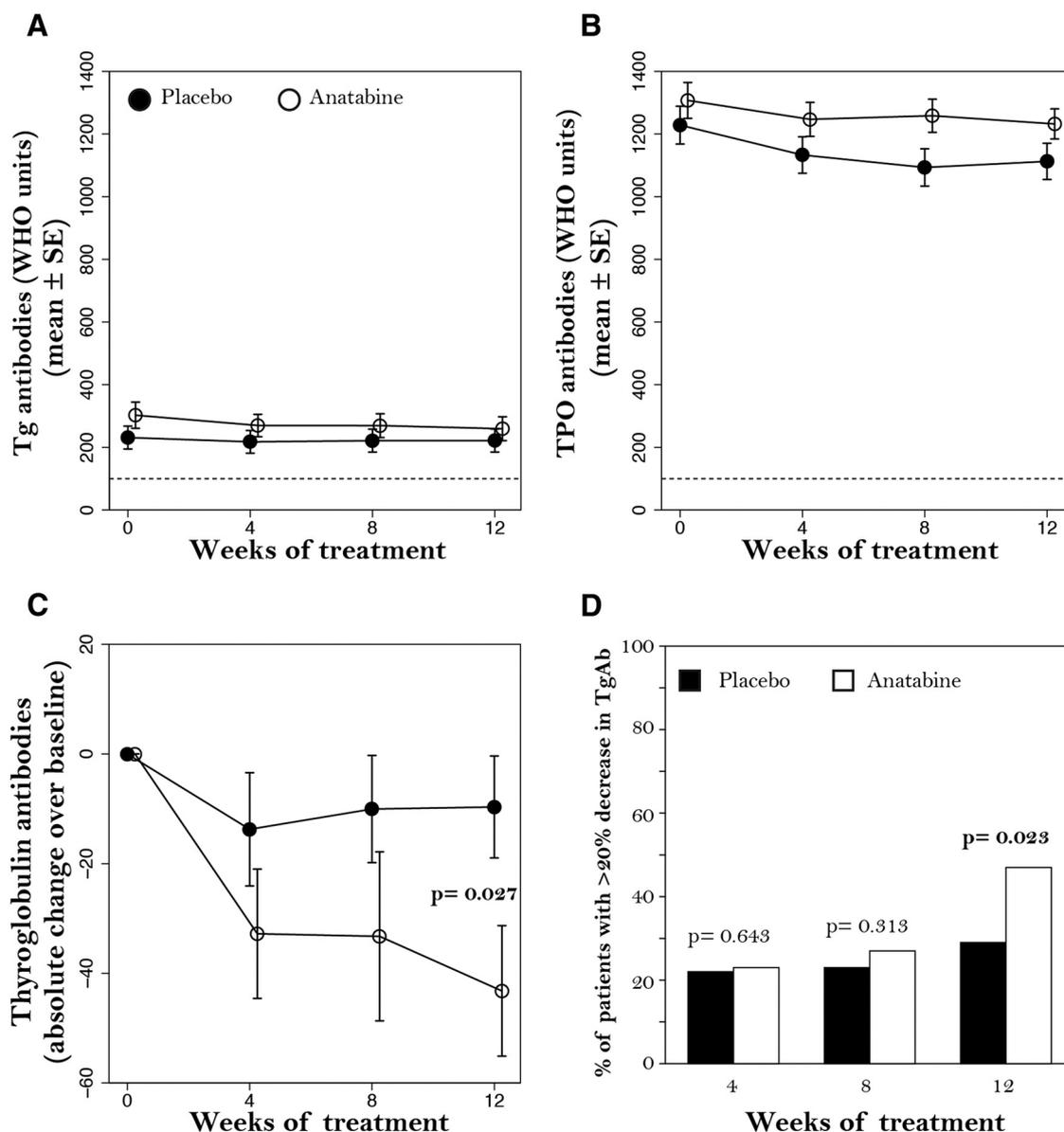
### Thyroid autoantibodies

The absolute change in mean TgAb levels between baseline and weeks 4, 8, and 12 revealed a significantly greater TgAb reduction in patients taking anatabine relative to those taking placebo by week 12 ( $P = .027$ , Figure 1C). TgAb values decreased by 46.2 ( $\pm 101.1$ ) WHO units

for the anatabine group and only  $3.9 (\pm 83.9)$  WHO units for placebo by week 12. The proportion of patients with a  $> 20\%$  decrease in TgAb was greater in the anatabine group (47%) than the placebo group (28%) at week 12 (Figure 1D,  $P = .023$ ). Due to significant heteroscedasticity in TgAb values, data were categorized based on reduction from baseline:  $\geq 25$ ,  $\geq 50$ ,  $\geq 75$ , and  $\geq 100$  WHO unit reductions). By week 12, the percentage of patients on anatabine with reductions in TgAb  $\geq 25$ ,  $\geq 50$ ,  $\geq 75$ , and  $\geq 100$  WHO units was significantly greater than in the placebo group for each category (all  $P < .05$ ; Supplemental Figure 2). No significant differences were found for TPOAb.

Subgroup analysis of patients taking levothyroxine

( $n = 36$ ) compared to those not ( $n = 34$ ) revealed a substantial decrease in TgAb from baseline at week 12 in the anatabine group on levothyroxine therapy ( $P = .008$ ). Additional analysis conducted with 31 patients in the anatabine group taking levothyroxine doses of  $\leq 1.0$  mcg/kg/d (range: 0.29 - 1.0 mcg/kg) revealed a greater effect of anatabine on TgAb levels. This analysis showed that decreases in TgAb from baseline at weeks 4, 8, and 12 were significant ( $P < .05$  for all). Further, there was a significant difference in mean TgAb between patients on anatabine and levothyroxine  $\leq 1.0$  mcg/kg/d vs those not on levothyroxine ( $-80.6$  vs.  $-23.1$  WHO units, respectively;  $P = .02$ ). No relationship between anatabine dose and TgAb lowering was observed.



**Figure 1.** Thyroglobulin (Tg) and thyroperoxidase (TPO) antibodies in patients treated with anatabine (open symbols) or placebo (closed symbols). Panels A and B depict the mean  $\pm$  standard error over time for Tg and TPO antibodies. Panel C shows the absolute change in Tg antibodies between subsequent visits and baseline. Panel D reports the percentage of patients with a  $> 20\%$  decrease in Tg antibodies.

## Serological thyroid function tests, inflammatory biomarkers, and thyroid ultrasonography

There were no significant changes or treatment group differences in serum thyroid function tests and inflammatory biomarkers, or ultrasonography measures (Supplemental Table 3).

## Adverse events and safety

More patients in the anatabine group (81%) reported AEs relative to the placebo group (44%,  $P < .05$ ) (Table 1). The most common AEs on anatabine were dizziness (36%), nausea (8%), and headaches (7%) during dose titration, and paresthesia (7%).

**Table 1.** Overall summary of adverse events for all patients who took at least one dose of their assigned study treatment ( $n = 165$ )

| Parameter <sup>a</sup>                        | Anatabine<br>( $n = 84$ )<br>n (%) | Placebo ( $n = 81$ )<br>n (%) |
|---|------------------------------------|-------------------------------|
| Adverse Event                                 | 68 (80.9)                          | 36 (44.4)                     |
| Treatment-Related Adverse Event <sup>b</sup>  | 52 (61.9)                          | 13 (16.0)                     |
| Most Commonly Reported Treatment-Related AEs: |                                    |                               |
| Dizziness                                     | 30 (35.7)                          | 2 (2.5)                       |
| Nausea  | 7 (8.3)                            | 2 (2.5)                       |
| Headache                                      | 6 (7.1)                            | 2 (2.5)                       |
| Paresthesia (tingling)                        | 6 (7.1)                            | 0                             |
| Insomnia                                      | 3 (3.6)                            | 0                             |
| Moderate or Severe Treatment-Related AE       | 23 (27.4)                          | 11 (13.6)                     |
| Serious Adverse Event <sup>c</sup>            | 1 (1.2)                            | 0                             |
| AE that Led to Withdrawal from Study          | 7 (8.3)                            | 1 (1.2)                       |
| Dose Reduction due to AE <sup>d,e</sup>       | 29 (34.5)                          | 2 (2.5)                       |
| Death outcome                                 | 0                                  | 0                             |

AE: Adverse event

<sup>a</sup> For each parameter, patients who had at least one event were included in the analysis; i.e. patients could have experienced multiple events within a particular parameter, but were only counted once.

<sup>b</sup> Determined by the Investigator as either possibly or probably related to study treatment.

<sup>c</sup> One patient in the anatabine group reported a serious adverse event (chest pain) that was considered unrelated to study treatment, did not result in withdrawal from the study, and did not recur following resumption of active study product.

<sup>d</sup> Anatabine dose reductions occurred with similar frequency regardless of dose group assignment, and occurred most often for patients prior to their week 4 site visit (i.e. the first in-person visit following initiation of dosing).

<sup>e</sup> Five of the patients in the anatabine group who had dose reductions subsequently withdrew from the study. The efficacy analysis group for anatabine ( $n = 70$ ) consisted of 24 (34%) patients who had a dose reduction, and 46 (66%) patients who completed the study at their maximum assigned dose.

Seven (8%) patients on anatabine and one (1%) on placebo withdrew from the study due to AEs; these were all considered mild or moderate. One patient on anatabine reported a serious AE, chest pain, which was evaluated and found to be of noncardiac origin, considered unrelated to study treatment, resolved without complications, and did not recur with resumption of anatabine.

There were no significant abnormalities in clinical laboratory values attributed to anatabine, and no clinically significant effects of either treatment on vital sign measures.

## Discussion

This study's results show a selective decrease in TgAb but not TPOAb in patients with Hashimoto's thyroiditis after 12 weeks of anatabine supplementation. Anatabine is an alkaloid found in plants of the *Solanaceae* family, including tobacco, tomatoes, potatoes, peppers, and eggplants (8). Although dietary anatabine's mechanism of action on thyroid autoimmunity remains to be elucidated, it may produce immunomodulatory effects through activation of alpha4beta2 or alpha7 cholinergic receptors like nicotine and other structurally related agonists (9, 10, 11). In a mouse model of thyroiditis, anatabine decreases thyroidal IL-1 beta and IL-18 levels (7), and in other experimental disease models it suppresses the inflammatory transcription factors STAT3 and NF-kB (12, 13, 14).

Multiple epidemiological studies have reported a protective effect of smoking on autoimmune hypothyroidism and thyroid antibodies (Supplemental Table 4). Noteworthy are studies showing selective effects of smoking on TgAb. Analysis of 4,125 randomly selected Danes found a negative association between smoking and presence of thyroid antibodies; the most pronounced association was found between smoking and TgAb, irrespective of TPOAb status (15). Analysis of a prospective population-based cohort of 9,362 pregnant mothers in Finland showed that mothers who smoked before pregnancy had lower TgAb prevalence than nonsmokers (2.5% vs 4.7%,  $P < .001$ ), whereas prevalences of TPOAb were similar (16).

Although TPOAb and TgAb are typically both measured for diagnostic purposes and often fluctuate in parallel with the disease course, the quantitative correlation between them is rather poor. Carlé et al (17) analyzed 145 patients with newly diagnosed autoimmune hypothyroidism and noted a correlation between TPOAb and TgAb ( $P < .001$ ), but a low Pearson's r-squared value (0.11). Analysis of NHANES data from 2007–2008 showed a similarly low r-squared value of 0.21 in more than 6,200 Americans (unpublished data).

A retrospective chart review study found that surgical removal or radioiodine ablation of the thyroid leads to gradual reductions in both TgAb and TPOAb over several years, with TgAb levels initially declining more rapidly than TPOAb (18). Based on those findings, it is possible that the differential response of TgAb and TPOAb that we observed after 12 weeks of treatment might have become less apparent following several additional months of anatabine supplementation, although that remains to be tested. It is also possible that anatabine specifically and directly affects thyroglobulin secretion resulting in a drop in TgAb production, as has been suggested regarding the effects of smoking on thyroid autoimmunity (15). Regardless of mechanism, anatabine in the diet reduced TgAb much like smoking, without nicotine or exposure to toxic smoke constituents.

Anatabine supplementation was safe and well tolerated. Patients in the anatabine group reported more AEs relative to the placebo group during upward dose titration, mostly mild nicotinic effects that resolved with dose adjustment.

The primary limitation to this study is its short duration. Longer treatment would be required to show a clinically meaningful effect on thyroid gland function and structure. Also, vitamin D is known to affect immune function (19), and the addition of this vitamin to both the active and placebo formulations may have obscured a treatment effect since TPOAb levels decreased in both groups. Third, the dose of anatabine may have been too low, although the amount taken per day (0.17–0.25 mg/kg) was expected to be safe and effective based upon case reports of individuals who experienced positive immunomodulatory effects within this dose range. Fourth, the inclusion of patients who were on levothyroxine could have influenced the results, as TgAb levels were lower in individuals in the anatabine group who were on levothyroxine therapy. Further studies are needed to see if anatabine could be adjunctive therapy to levothyroxine, or if its effects would be more or less robust without the addition of vitamins A and D3. Finally, because urinary iodide measurements were not performed, a potential effect of dietary iodine status on anatabine responsiveness could not be assessed.

This study shows nutritional supplementation with anatabine significantly reduces circulating TgAb levels in Hashimoto's thyroiditis patients. These results confirm an immunologic effect of anatabine in humans as previously shown in a preclinical model of Hashimoto's thyroiditis (7). Collectively, the results of this trial suggest that further studies of anatabine in Hashimoto's thyroiditis and other autoimmune disorders, including studies that monitor

above changes for longer time periods and elucidate its mechanism of action, are warranted.

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