

## Original Article

# Beneficial therapeutic effects of a Chinese herbal formula on autoimmune thyroiditis: preclinical results and outcome of a pilot clinical study

Hang Ren<sup>1\*</sup>, Wei-Ni Chen<sup>2\*</sup>, Ya-Hui Liu<sup>1</sup>, Hui-Fang Shen<sup>1</sup>, Guo-Zhen Cui<sup>1</sup>

<sup>1</sup>Zhuhai Key Laboratory of Basic and Applied Research in Chinese Medicine, Department of Bioengineering, Zhuhai Campus of Zunyi Medical University, Zhuhai, Guangdong, China; <sup>2</sup>The Second People's Hospital of Zhuhai, Zhuhai, Guangdong, China. \*Equal contributors.

Received February 26, 2018; Accepted June 29, 2018; Epub December 15, 2018; Published December 30, 2018

**Abstract:** Objective: Hashimoto's thyroiditis (HT), is a chronic autoimmune disease characterized by variable degrees of lymphocytic infiltration and thyroid gland destruction, often leading to hypothyroidism. This purpose of the current study was to investigate the effects of a herbal formula named Buqi Huatan (BQHT) on HT. Methods: The current study involved experimental and clinical investigations. For experimental investigation, experimental autoimmune thyroiditis (EAT) mice were treated with vehicle or various doses of the herbal formula by oral administration once daily for 8 weeks (n = 8 per group). Thyroid function was evaluated by assessing the following parameters: thyroid histopathology, serum levels of thyroid-stimulating hormone (TSH), free thyroxine (FT4), thyroid autoantibodies (TPOAb and TgAb), and inflammatory cytokines. For clinical investigation, therapeutic effects of BQHT combined with levothyroxine (LT4) for treatment of patients with Hashimoto's thyroiditis were observed according to clinical guidelines. Results: In EAT mice, compared with the vehicle group, the level of FT4 was significantly increased while TSH, TPOAb, and TgAb were reduced in the treatment groups ( $P < 0.05$ ). Thyroid histopathology analysis demonstrated that BQHT protected the integrity of the thyroid cell and decreased the extent of lymphocyte infiltration. It also regulated the immune response to obtain a balance, which reversed the levels of interleukin-10 (IL-10) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) both in serum and thyroid tissue of EAT mice. In Hashimoto's thyroiditis patients, compared with the LT4 only group, combined BQHT with LT4 treatment significantly decreased TSH and enhanced FT4, and slightly suppressed the titers of TPOAb and TgAb. Conclusion: The results from experimental animal and pilot clinical studies indicate that formula BQHT displayed potential therapeutic effects against HT, and this study provides a basis for the further development of Chinese medicine formula BQHT.

**Keywords:** Hashimoto's thyroiditis, traditional chinese medicine, BQHT, IL-10, TNF- $\alpha$

## Introduction

Hashimoto's thyroiditis (HT), is one chronic autoimmune disease characterized by variable degrees of lymphocytic infiltration and thyroid gland destruction, often leads to hypothyroidism, a condition in which thyroid loses its ability to produce enough thyroid hormones for the body's use. HT is frequently diagnosed between the ages of 30 and 50 years [1]. The prevalence is 8 cases per 1000, female is at least 8 times more likely than male to have HT [2]. HT is the most frequent cause of hypothyroidism [3]. Moreover, recent clinical studies have demonstrated that chronic autoimmune conditions of

thyroid increase the prevalence of thyroid cancer in HT patients [4, 5].

Diagnosis of HT is based on thyroid dysfunction, an enlarged thyroid gland and detection of serum thyroid peroxidase (TPO) and thyroglobulin (Tg) autoantibodies, of which TPO is more important [2]. Currently, levothyroxine (LT4), a man-made T4, has been considered the standard treatment method for HT [6]. However, this thyroid hormone replacement management has some drawbacks. For instance, patients have to take it for a lifetime and adjust the dose as necessary, typically based on the levels of thyroid hormones. Even if the total replacement

## Effects of a Chinese herbal formula on autoimmune thyroiditis

dose is reached, recent investigations suggest that LT4 cannot keep a euthyroid state [7]. Available clinical evidences indicated that patients with HT under LT4 replacement therapy didn't have the improvement in the cognitive and psychological well-being function [8-10]. Thus, developing new therapies for HT patients to avoid this kind of limitation of hormone replacement therapy remains a major challenge.

The traditional Chinese medicine (TCM) has been applied for a long history and proven successful treatment for chronic diseases in China and some other Asian countries. A good case is evidence for the relationship between TCM and chronic cardiovascular disease. A systematic analysis of 68 random clinical trials, including a total of 16,171 patients indicated that TCM was significantly effective in improving hypertension and heart diseases. The risk of adverse effects was similar to that of no intervention, placebo, or western medications [11]. The results of double-blinded randomized clinical trial study demonstrated that one Chinese herbal formula RYJGT significantly decrease serum hepatitis C virus RNA level compared to a placebo group in chronic hepatitis C patients [12]. Here, a new herbal formula named Buqi Huatan (BQHT) was the result of Dr. Guozhen Cui in collaboration with TCM physicians. This formula was made according to TCM theories, ancient prescriptions, and rich clinical experience. In this study, HT was investigated to evaluate the therapeutic effects of BQHT on thyroid function in both EAT mice and HT patients.

### Materials and methods

#### *The preparation of BQHT*

BQHT contains 6 individual herbs, namely, *Astragalus membranaceus* (Huangqi), *Figwort Root* (Xuanshen), *Rehmanniae Radix Praeparata* (Shudihuang), *Chuanxiong Rhizoma* (Chuanxiong), *Fritillariae Thunbergii Bulbus* (Zhebeimu), *Poria Cocos* (Schw) Wolf (Fuling). All herbs used in this study were commonly commercially available dry matter and purchased from Yuan-chunlin Pharmacy Company Limited (Zhuhai, China). The herbs were identified by Dr. Guozhen Cui (Biological Engineering Department, Zunyi Medical University, China). The voucher specimen (number ZMC11) was deposited in Zhuhai key laboratory of fundamental and applied

research in traditional Chinese medicine. The BQHT extract was prepared as described in the patent No. 201710745413.6. Briefly, the six herbs were ground into fine powders and mixed in the ratio of 3:1:1:1.5:1.5:1.5 (w/w), then soaked in 60% ethanol about 10 volumes at room temperature for overnight. They were heated to 60°C and maintained for 2 hours, then filtered through 8 layers of gauze. The residue was repeated two cycles by the same procedure [13, 14]. Finally, the extract was freeze-dried and stored at 4°C until further use. The extraction yield was approximately 21% (w/w, dried extract/crude herb). Quality control was performed by high performance liquid chromatography (HPLC) based on the method described in "Chinese Pharmacopoeia" (data not shown).

#### *Preparation of EAT model and treatment*

Female Kunming mice at 6-8 weeks age were provided by Guangdong Medical Laboratory Animal Center (License No. SCXK 2013-0002). The animals were housed under specific-pathogen-free (SPF) standard conditions at indoor temperature  $23 \pm 1^\circ\text{C}$  and humidity of  $55 \pm 5\%$  with automatic light-dark cycle (12 hour-12 hour). All experiments procedures were carried out in accordance with the Guidelines established by the guide for the Care and Use of Laboratory Animals of Zunyi Medical University. All animals were obtained care on the basis of the Guide for the Care and Use of Laboratory Animals (NIH).

After one-week of adaptive feeding, 24 mice were immunized five times with porcine thyroglobulin (PTg, Sigma, St Louis, MO, USA) according to previous studies [15]. The remaining eight mice were used as controls. For the induction of EAT model, 80 µg PTg was emulsified in 80 µl complete Freund's adjuvant (CFA, Sigma-Aldrich, St Louis, MO, USA) or incomplete Freund's adjuvant (IFA, Sigma-Aldrich, St Louis, MO, USA). The PTg emulsion was then injected into each mouse subcutaneously at multiple sites on day 0 and 7th day. In the following three weeks, the injection of PTg was delivered by the same method. In addition, the control group was injected with saline instead of PTg emulsion. Furthermore, EAT mice were given water containing 0.05% (w/v) NaI (Aladdin, Shanghai, China) daily during the entire process [16]. Mice in the BQHT treatment groups

## Effects of a Chinese herbal formula on autoimmune thyroiditis

**Table 1.** Baseline characteristics of the 28 patients

Features	LT4 (n = 19)	LT4+BQHT (n = 9)
Age, mean (SD), years	34.3 ± 11.0	33.4 ± 9.7
Gender	18 women 1 man	8 women 1 man

were orally administered once daily 0.5 g/kg or 1.0 g/kg of BQHT for 8 weeks. The controls and models were treated with an equal volume vehicle.

### Biochemical analysis

The mice were sacrificed on day 56 after treatment. Blood samples were obtained and centrifuged at 3000 g/min for 20 minutes. Serum was separated for examination of FT4, TSH, TPOAb, and TgAb by chemiluminescent immunoassay (CLIA) using the UniCel™ DxI 800 Access Immunoassay System (Beckman Coulter Inc., Fullerton, CA, USA) according to the manufacturers' instructions.

### Histopathology of thyroid gland in EAT mice

Thyroids were graded by hematoxylin and eosin (HE) staining for histological analysis. The extent of lymphocytic cell infiltration was observed under a light microscope (BX43, Olympus, Tokyo, Japan) and then scored in a blinded fashion. The severity of thyroiditis was graded as previously described [17, 18], based on the percentage of thyroid infiltrated: score 0, naïve thyroid; score 1, less than 1% lymphocytic infiltration of the thyroid; score 2, 1-9% lymphocytic infiltration; score 3, 10-40% lymphocytic infiltration; score 4, more than 40% lymphocytic infiltration.

### Detection of IL-10 and TNF- $\alpha$ levels in EAT mice

Serum IL-10 and TNF- $\alpha$  levels were determined by quantitative sandwich ELISA Kits (Cusabio Biotech, Wuhan, China) according to the manufacturer's protocol. IL-10 and TNF- $\alpha$  expression levels in the thyroid tissue were detected by immunohistochemical staining using the corresponding immunohistochemical detection kits (Bioss, Peking, China) according to the manufacturer's instruction. The positive cells showed yellow or brown articles in the cytoplasm with a blue colored cellular nucleus. The mean optical density (MOD) reflected the protein expression in immunohistochemical staining. The MOD,

refers to density (IOD/area) of each field, was evaluated using Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) and is shown normalized to the control group.

### Clinical study design and patient enrollment

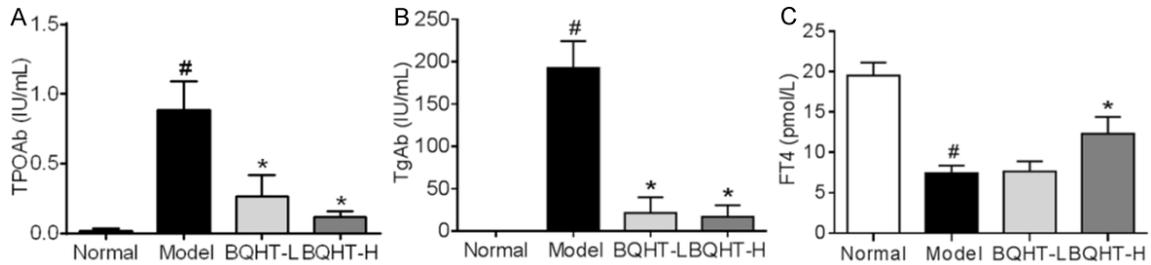
In order to further investigate the benefits of treating HT patients with BQHT plus LT4 combination therapy compared to LT4 monotherapy, an open-label non-randomized pilot clinical study was performed from March 2016 to February 2017. The HT patients involved in this study were recruited from the endocrinology outpatient clinic of the Second People's Hospital of Zhuhai. Patients were included when a biochemically confirmed diagnosis of HT was established. HT diagnosis was defined by the presence of the following criteria for at least 3 months [2]: (1) elevated serum TPOAb (>100.0 IU/mL); (2) raised serum TSH level (>4.0 mIU/L). Patients were eligible for the trial if they were between the ages of 18 and 60 years old, and excluded if they had any other clinically relevant conditions that might be associated with thyroid dysfunction, such as if they were pregnant, had thyroid cancer, previous radioiodine treatment, or previous thyroid surgery. The Ethics Committee of the Second People's Hospital approved the study procedure which was carried out according to the Declaration of Helsinki. All subjects were provided with and agreed to written, informed consent for their participation.

Patients were allocated to receive LT4 alone or combined LT4 plus BQHT (10 g) once daily treatment. Treatment duration was 8 weeks. The dose of LT4 was assigned based on the serum TSH values as described [19]. The Pharmacy Department of the hospital supervised the production, dispensing, and storage of LT4 and formula BQHT. Baseline characteristics of the patients were obtained at the pre-treatment visit (**Table 1**). The patients were assigned to combined BQHT plus LT4 or LT4 alone treatment according to their willingness. Serum TSH, FT4, TPOAb, and TgAb levels were measured as described above.

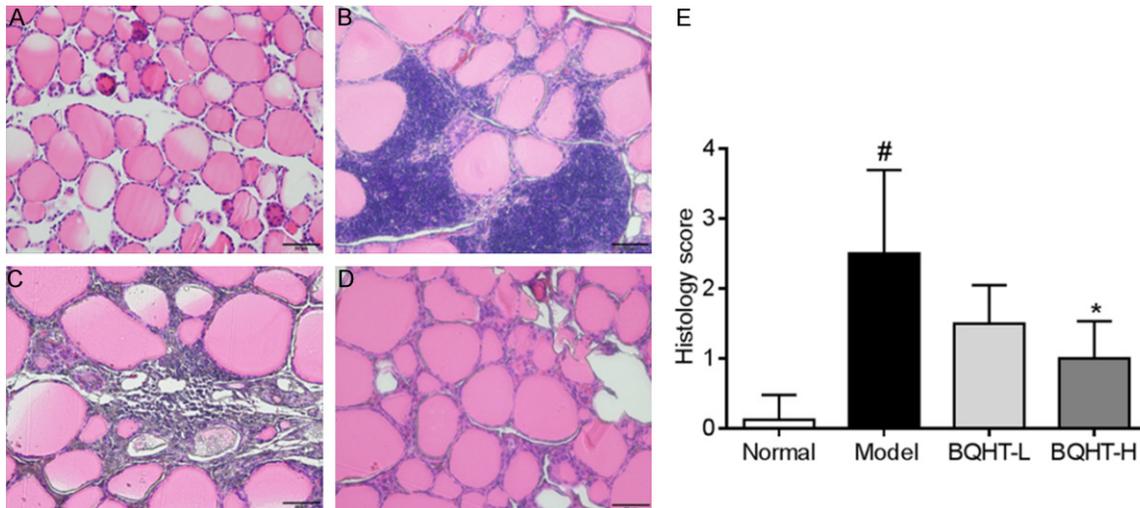
### Statistical analysis

Differences between groups were compared by one-way analysis of variance (*one-way ANOVA*). For statistical analysis of clinical data, to evaluate the change of TSH, FT4 TPOAb, and TgAb

## Effects of a Chinese herbal formula on autoimmune thyroiditis



**Figure 1.** Effects of BQHT on thyroid function in EAT mice. Levels of TPOAb (A), TgAb (B) and FT4 (C) in the serum of EAT mice were evaluated. <sup>#</sup> $P < 0.05$  compared with normal control group, <sup>\*</sup> $P < 0.05$  compared with EAT model group.



**Figure 2.** Effects of BQHT on thyroid glands pathology of EAT in mice. Representative histological sections of thyroid glands stained with HE in normal control (A), EAT (B), low dose of BQHT (C) and high dose of BQHT (D). Scale bar: 50  $\mu$ m. Histological scores were blindly scored using HE stained thyroid tissue sections (E). <sup>#</sup> $P < 0.05$  compared with normal control group, <sup>\*</sup> $P < 0.05$  compared with model group.

concentrations in patients before and after treatment, paired t-tests were used. All calculations were performed using SPSS software (version 17, SPSS Inc., Chicago, IL, USA). Data are shown as mean  $\pm$  SD. A value of  $P < 0.05$  was considered significant.

### Results

#### Thyroid function in EAT mice

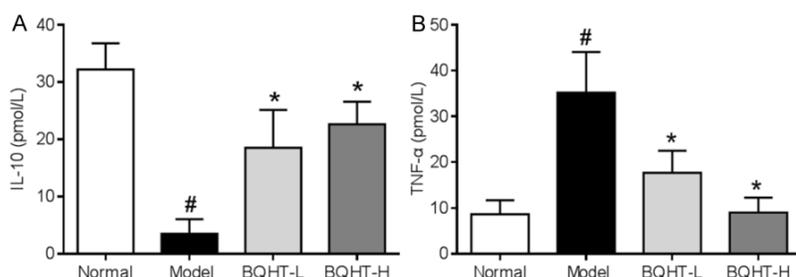
First, to evaluate the effects of formula BQHT on the development of EAT in mice, serum levels of TgAb, TPOAb, and FT4 were measured. As shown in **Figure 1**, compared with the normal group, serum levels of TgAb and TPOAb were significantly higher while the FT4 level was obviously lower ( $P < 0.05$ ). These results indicated that EAT model in mice was induced successfully. Over a period of 8 weeks of treatment, the serum levels of both autoantibodies, TPOAb

and TgAb were significantly decreased compared with the model group in a dose-dependent manner (**Figure 1A, 1B**). Importantly, oral administration of high dose BQHT (1.0 g/kg) significantly increased the serum FT4 level compared with the model group ( $P < 0.05$ ).

#### Histopathology of the thyroid gland in EAT mice

The presence of interstitial lymphocyte infiltration of the thyroid gland is one feature of autoimmune thyroiditis, thus lymphocyte infiltration was determined by histopathological analysis. As shown in **Figure 2B, 2E**, the histopathology score was significantly increased in the model group compared with the normal control group, thus once again indicating that thyroiditis was successfully induced in mice. BQHT protected the integrity of the thyroid cell and decreased the extent of lymphocyte infiltration. In contrast, oral administration of high dose BQHT

## Effects of a Chinese herbal formula on autoimmune thyroiditis



**Figure 3.** Effects of BQHT on serum levels of IL-10 and TNF- $\alpha$  in EAT mice. Serum concentrations of IL-10 (A) and TNF- $\alpha$  (B) were determined by ELISA method. # $P < 0.05$  versus normal control group, \* $P < 0.05$  versus model group.

(1.0 g/kg) significantly decreased the score of histopathological damage in thyroids compared with model group ( $P < 0.05$ ), and it was not reduced significantly in the low dose of BQHT group ( $P > 0.05$ ). In addition, in the BQHT treatment groups the thyroid tissue maintained structural integrity relative to the model, with uniform, round, or oval thyroid epithelial cells arranged in well-ordered rows (Figure 2C, 2D). The histological scores were significantly lower in BQHT treated mice (Figure 2E). These results indicate that the severity of thyroiditis, assessed by the abundance of lymphocyte infiltration in EAT mice, was significantly reduced after treatment with BQHT.

### IL-10 and TNF- $\alpha$ levels in EAT mice

The cytokines IL-10 and TNF- $\alpha$  are important in autoimmune thyroiditis. Accordingly, we determined whether the levels of the both cytokines were changed. The results of ELISA assay demonstrated that the level of serum IL-10 was significantly decreased while that of TNF- $\alpha$  was enhanced in model group compared with those in normal control group. Conversely, oral administration of BQHT at a dose range from 0.5 to 1.0 g/kg significantly reversed the both serum cytokine levels compared with the model group in a dose-dependent manner ( $P < 0.05$ , Figure 3). Similar results were observed immunohistochemically in the thyroid gland where BQHT significantly enhanced IL-10 protein expression level while reduced that of TNF- $\alpha$  ( $P < 0.05$ , Figure 4).

### Baseline characteristics of HT patients

Twenty-eight patients met our enrolling criteria and were divided into either LT4 group ( $n = 19$ ) or LT4 combined with BQHT group ( $n = 9$ ). The

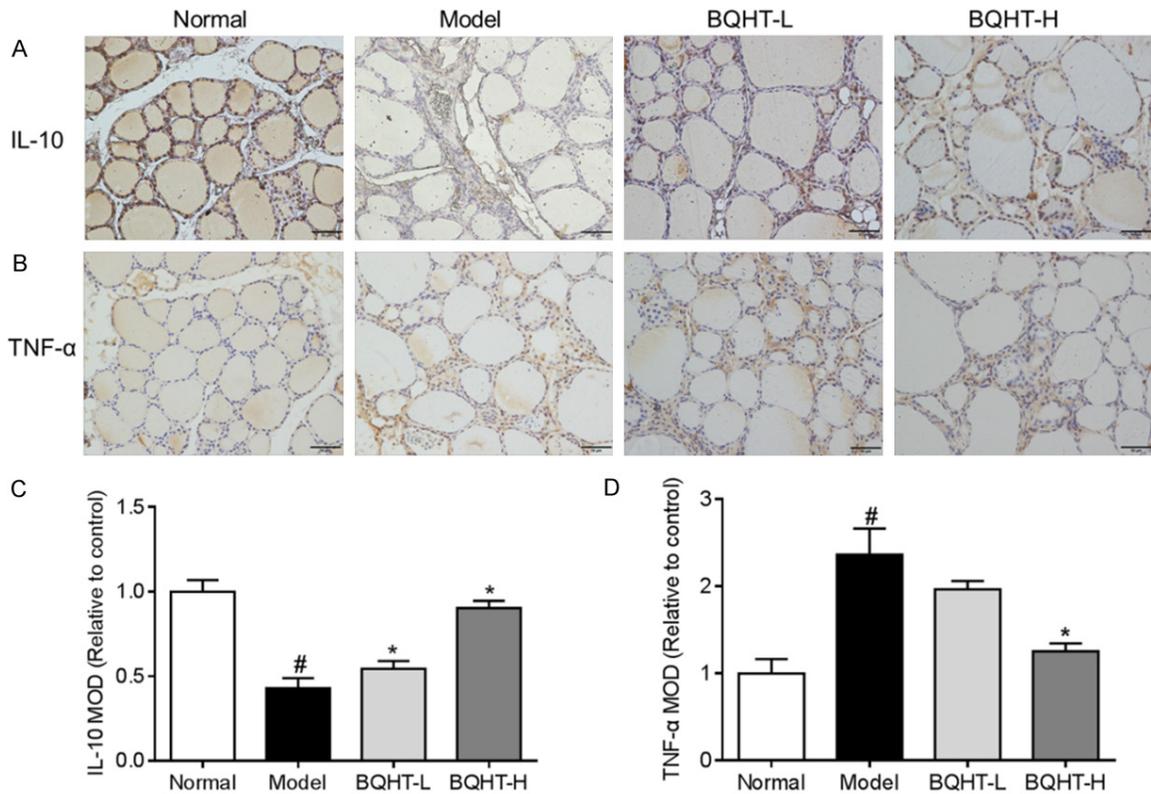
levels of TSH and FT4 were tested as the primary outcomes. Serum TPOAb and TgAb were the secondary outcomes as the indicators for the autoimmune thyroid. All non-randomized patients underwent 8 weeks BQHT plus LT4 or LT4 only treatment. All the analysis were performed before and after drug treatment, and were included in the final statistical analysis.

During this period, the patients did not change any medications such as LT4 dosage that would affect the study outcomes. No adverse effects were observed in either group during the study. No significant differences in key parameters, such as the levels of thyroid hormones, LT4 dosage and ages, between the two groups before the treatment were found (Table 1).

### Thyroid function in HT patients

In order to further investigate the effect of BQHT on thyroid function in HT patients, serum levels of TSH, FT4, TPOAb, and TgAb were determined. Figure 5 compared the changes from baseline to 8 weeks in the four serum biochemical levels. As expected, LT4 alone treatment group, the serum TSH level was decreased, and the serum FT4 was increased, but it was not significant ( $P > 0.05$ , Figure 5A, 5B). Of note, serum TPOAb and TgAb levels were similar before and after treatment. In contrast, in the combined therapy group, the changes in TSH and FT4 were significantly comparative different between the study groups ( $P < 0.05$ ). After 8 weeks combined drug treatment, serum TSH concentration decreased from  $18.50 \pm 9.02$  mIU/L to  $2.75 \pm 0.82$  mIU/L (Figure 5A), FT4 concentration increased from  $8.15 \pm 0.75$  pmol/L to  $11.55 \pm 0.76$  pmol/L (Figure 5B). Since the LT4 dosage remained the same for all the enrolled patients, the increased FT4 and the decreased TSH results strongly suggested improved thyroid function. TPOAb and TgAb levels were tested to confirm whether BQHT regulated the autoimmune conditions in HT patients. Indeed, 8 weeks combined treatment slightly reduced the level of TPOAb from  $659.82 \pm 139.26$  IU/mL to  $524.45 \pm 109.10$  IU/mL (Figure 5C), and TgAb from  $603.80 \pm 305.88$  IU/mL to  $486.37 \pm 259.83$  IU/mL (Figure 5D).

## Effects of a Chinese herbal formula on autoimmune thyroiditis



**Figure 4.** Effects of BQHT on expression of IL-10 and TNF- $\alpha$  in thyroid tissues. Protein expression of IL-10 (A, C) and TNF- $\alpha$  (B, D) in thyroid gland tissues were measured by IHC assay. Microscope images were representative and semi-quantification data were mean with SD from 8 mice in each group. Scale bar: 50  $\mu$ m. # $P$  < 0.05 versus normal control group, \* $P$  < 0.05 versus model group.

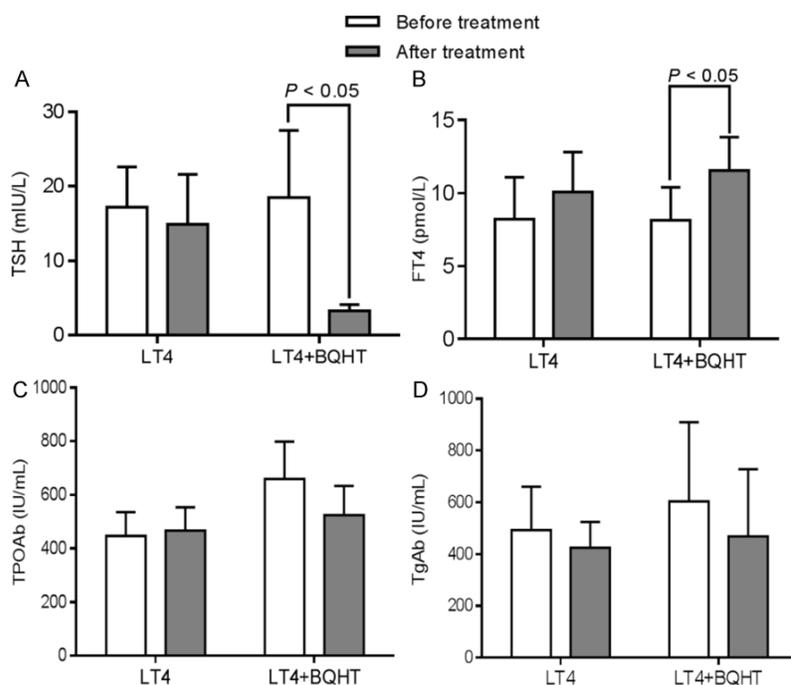
### Discussion

This study highlights the interesting pharmacological action of a herb formula BQHT, against autoimmune thyroiditis both in experimental and clinical investigations. An intriguing finding of our study is that BQHT has beneficial therapeutic effects, leading to the enhanced thyroid function, decreased degree and the range of thyroid lymphocyte infiltration in EAT mice. In clinical investigation, combined BQHT with LT4 treatment significantly decreased TSH level and enhanced FT4 compared with the common thyroid hormone replacement therapy, LT4. The use of LT4 has prominent defects, including lifetime drug use, dose adjustment every 4-6 weeks. The objectives of treating hypothyroidism are to alleviate the symptoms of hypothyroidism, restore euthyroidism in body tissues, and normalize serum TSH, which is a surrogate marker for thyroid hormone action at the tissue level. In contrast, our preclinical animal results have demonstrated that BQHT treatment offers advantages in these aspects over the tradition-

al hormone replacement therapy. Hence, BQHT may be an effective and alternative therapeutic approach for the treatment of autoimmune thyroiditis.

The process of autoimmune thyroiditis is complex, typically consisted of the enhanced titer of autoantibodies and TSH, leading to chronic thyroid inflammation. The precise pathogenic mechanisms of autoimmune thyroiditis have not been fully clarified but are likely to be multifactorial. Savas et al. reported patients with thyroid dysfunctions had higher serum inflammation markers than the control subjects, which revealed that inflammation played a vital part in thyroid pathogenesis [20]. In particular, immunosuppressive cytokine IL-10 is purportedly critical element underlying the autoimmune thyroiditis [21, 22]. The results from IL-10 knockout mice suggested that IL-10 deficiency enhanced the susceptibility to EAT [23]. Consistent with this preclinical study, dysfunction of Breg cells, expressing IL-10, in patients with HT is associated with the downregulation of

## Effects of a Chinese herbal formula on autoimmune thyroiditis



**Figure 5.** Clinical efficacy of BQHT on thyroid function in patients with Hashimoto's thyroiditis. Thyroid function in the patients before and after treatment with LT4 or BQHT+LT4 for 8 weeks, characterized by the serum levels of TSH (A), FT4 (B), TPOAb (C) and TgAb (D).

IL-10 [24]. In line with these studies, our results obtained by both ELISA and IHC methods demonstrated that BQHT significantly reversed the decreased IL-10 level in serum and thyroid tissue of EAT mice (Figures 3A, 4A, 4C). In addition, BQHT was found to significantly suppress the serum levels of TPOAb and TgAb (Figure 1), but not total IgG (data not shown). These results indicate that BQHT regulates the immune response to acquire balance between normal immune response and development of autoimmune thyroiditis. These results suggest that BQHT, at least in part, acts directly or indirectly through IL-10-mediated immune regulation.

Besides the enhancement of IL-10 generation, other molecular mechanisms, such as inhibition of the excessive TNF- $\alpha$ , a major pro-inflammatory cytokine involved in inflammatory diseases [25, 26]. Furthermore, significant positive correlation was found between TNF- $\alpha$  and TPOAb levels [27]. It has been implicated in the therapeutic interventions against the progressive of autoimmune diseases, where anti-TNF- $\alpha$  biological agents have been successfully used to treat patients with autoimmune inflammatory diseases, such as rheumatoid arthritis,

Crohn's disease, and ankylosing spondylitis [28-30]. The findings from the current study showed that the levels of TNF- $\alpha$  both in serum and thyroid tissue were significantly increased and treatment with BQHT significantly reduced TNF- $\alpha$  generation in EAT mice (Figures 3B, 4B, 4D). Collectively, the reduced TNF- $\alpha$  production appears to play a pivotal role in the phase of therapeutic beneficial effects of BQHT in EAT mice.

In the clinical aspect, we noticed an improvement in thyroid function after 2 months of administration of BQHT with LT4 in patients with HT. This improvement was statistically significant in the serum levels of TSH and FT4 ( $P < 0.05$ ). Furthermore, we also

found that this combined treatment had a trend towards lower titers of TPOAb and TgAb than those in LT4 only treatment group, despite not reaching statistical significance ( $P > 0.05$ ). It was well known that TPOAb is an important index for evaluating the thyroid autoimmune conditions related to the risk of thyroid cancer in HT patients [31]. For instance, recent studies showed that autoimmunity impacts on health-related quality of life in patients with autoimmune hypothyroidism, especially in terms of psychological symptoms [32, 33].

Perhaps significant results in lowering the autoantibodies would be seen if the treatment duration was extended. In addition, no patients developed side effects during BQHT treatment. These findings indicated that BQHT is effective and safe when treating patients with HT.

Our results are compatible with those reported by Song et al. who showed that a herbal formula, modified Haizao Yuhu Decoction, conferred significant protective effects by reversing the levels of thyroid hormone and thyroid-related autoantibodies in EAT rat [16]. Similar to our clinical data, Ding et al. showed that Chinese medicine Chaihushugan decreased TSH and

## Effects of a Chinese herbal formula on autoimmune thyroiditis

autoantibody level significantly, therefore enhanced the clinical efficacy and quality of life [34]. Also, Sa et al. found that herbal medicine Gamgungtang down-regulated autoreactivity via maintaining Th1/Th2 lymphocyte balance in EAT mice [35]. By combining this reported literature and our current results, it seems that herbal medicine not only reversed the thyroid function but also regulated the immune balance, which regulated the both functions leading to the beneficial effects against autoimmune thyroiditis.

Since in our clinical study, the treatment is only short-term and combined use of BQHT with LT4, the effects of the long-term and/or BQHT only treatment are needed to observe its further therapeutic benefits in a larger number of HT patients. Sample sizes in both groups are small and the further larger scale randomized clinical trials should be conducted in the future.

In conclusion, our current study demonstrates that BQHT is effective on reversing thyroid function and reducing the autoimmune responses in both EAT animal model and HT patients. The therapeutic effects of BQHT are mediated, at least in part, through improving the IL-10/TNF- $\alpha$  cytokine balance. In addition, combined BQHT with LT4 therapy is a promising new approach in treating HT patients.

### Acknowledgements

This work was supported by the startup fund of Zunyi Medical University (Grant No. F-910) and Premier-Discipline Enhancement Scheme Supported by Zhuhai Government Fund.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Guo-Zhen Cui, Zhuhai Key Laboratory of Basic and Applied Research in Chinese Medicine, Department of Bioengineering, Zhuhai Campus of Zunyi Medical University, Jinwan District, Zhuhai 519041, China. Tel: 86-756-7623310; Fax: 86-756-7623310; E-mail: cgzum@hotmail.com

### References

[1] Parvathaneni A, Fischman D and Cheriya P. Hashimoto's thyroiditis. *Fukuleaks Org* 2012; 21: 173-175.

- [2] Caturegli P, Remigis AD and Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmun Rev* 2014; 13: 391-397.
- [3] Tandon N. Management of hypothyroidism in adults. *J Assoc Physicians India* 2011; 59 Suppl: 21-25.
- [4] Ehlers M and Schott M. Hashimoto's thyroiditis and papillary thyroid cancer: are they immunologically linked? *Trends Endocrinol Metab* 2014; 25: 656-664.
- [5] Paparodis R, Imam S, Todorova-Koteva K, Staii A and Jaume JC. Hashimoto's thyroiditis pathology and risk for thyroid cancer. *Thyroid* 2014; 24: 1107-1114.
- [6] Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS and Sawka AM. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid* 2014; 24: 1670-1751.
- [7] Wiersinga WM. Paradigm shifts in thyroid hormone replacement therapies for hypothyroidism. *Nat Rev Endocrinol* 2014; 10: 164-174.
- [8] Giannouli V, Toulis KA and Syrmos N. Cognitive function in Hashimoto's thyroiditis under levothyroxine treatment. *Hormones (Athens)* 2014; 13: 430-433.
- [9] Saravanan P, Chau WF, Roberts N, Vedhara K, Greenwood R and Dayan CM. Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. *Clin Endocrinol* 2002; 57: 577-585.
- [10] Kelderman-Bolk N, Visser TJ, Tijssen JP and Berghout A. Quality of life in patients with primary hypothyroidism related to BMI. *Eur J Endocrinol* 2015; 173: 507-515.
- [11] Hao PP, Jiang F, Chen YG, Yang J, Zhang K, Zhang MX, Zhang C, Zhao YX and Zhang Y. Traditional Chinese medication for cardiovascular disease. *Nat Rev Cardiol* 2015; 12: 115-122.
- [12] Chen FP, Chang CM, Wu TP, Yang JL, Kung YY, Huang YH, Su CW, Lan KH, Chiang SC and Hwang SJ. Clinical efficacy of Rong-Yang-Jyh-Gan-Tang on patients with chronic hepatitis c: a double-blinded randomized placebo-controlled crossover study. *J Ethnopharmacol* 2017; 196: 1-8.
- [13] Gang C, Yang Y, Hu C, Cheng X, Xu Y, Cai X, Min W, Yang CS and Peng C. Protective effects of Huangqin Decoction against ulcerative colitis and associated cancer in mice. *Oncotarget* 2016; 7: 61643-61655.
- [14] Chen Y, Xian Y, Lai Z, Loo S, Chan WY and Lin ZX. Anti-inflammatory and anti-allergic effects and underlying mechanisms of Huang-Lian-Jie-Du extract: implication for atopic dermatitis

## Effects of a Chinese herbal formula on autoimmune thyroiditis

- treatment. *J Ethnopharmacol* 2016; 185: 41-52.
- [15] Ma S, Chen X, Wang L, Wei Y, Ni Y, Chu Y, Liu Y, Zhu H, Zheng R and Zhang Y. Repairing effects of ICAM-1-expressing mesenchymal stem cells in mice with autoimmune thyroiditis. *Exp Ther Med* 2017; 13: 1295-1302.
- [16] Song XH, Zan RZ, Yu CH and Wang F. Effects of modified haizao yuhu decoction in experimental autoimmune thyroiditis rats. *J Ethnopharmacol* 2011; 135: 321-324.
- [17] Bonita RE, Rose NR, Rasooly L, Caturegli P and Burek CL. Kinetics of mononuclear cell infiltration and cytokine expression in iodine-induced thyroiditis in the NOD-H2h4 mouse. *Exp Mol Pathol* 2003; 74: 1-12.
- [18] Mori K, Yoshida K, Tani J, Nakagawa Y, Hoshikawa S, Ozaki H and Ito S. Effects of angiotensin II blockade on the development of autoimmune thyroiditis in nonobese diabetic mice. *Clin Immunol* 2008; 126: 97-103.
- [19] Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA and Woeber KA. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the american association of clinical endocrinologists and the american thyroid association. *Endocr Pract* 2012; 18: 988-1028.
- [20] Savas E, Sahin AZ, Aksoy SN, Tascan A, Sayiner ZA and Ozkaya M. Serum levels of inflammatory markers in patients with thyroid dysfunction and their association with autoimmunity status. *Int J Clin Exp Med* 2016; 9: 4485-4490.
- [21] Wakkach A, Augier S, Breitmayer JP, Blin-Wakkach C and Carle GF. Characterization of IL-10-secreting T cells derived from regulatory CD4+CD25+ cells by the TIRC7 surface marker. *J Immunol* 2008; 180: 6054-6063.
- [22] Saraiva M and O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010; 10: 170-181.
- [23] Yu Z, Liu T, Liu S, Zou H, Sun X, Shi X, Li Y, Shan Z and Teng W. Interleukin-10 influences susceptibility to experimental autoimmune thyroiditis independently of the H-2 gene. *Int J Mol Med* 2015; 35: 413-424.
- [24] Yu S, Qi Y, Wang H, Jiang J, Sun L and Zhou Q. Dysfunction of CD24+CD38+ B cells in patients with hashimoto's thyroiditis is associated with a lack of interleukin 10. *Int J Biochem Cell Biol* 2017; 90: 114-120.
- [25] Ellis JS and Bralemullen H. Mechanisms by which B cells and regulatory T cells influence development of murine organ-specific autoimmune diseases. *J Clin Med* 2017; 6: 13.
- [26] Wang SH, Bretz JD, Phelps E, Mezosi E, Arscott PL, Utsugi S and Baker JR Jr. A unique combination of inflammatory cytokines enhances apoptosis of thyroid follicular cells and transforms nondestructive to destructive thyroiditis in experimental autoimmune thyroiditis. *J Immunol* 2002; 168: 2470-2474.
- [27] Mikos H, Mikos M, Rabska-Pietrzak B and Niedziela M. The clinical role of serum concentrations of selected cytokines: IL-1beta, TNF-alpha and IL-6 in diagnosis of autoimmune thyroid disease (AITD) in children. *Autoimmunity* 2014; 47: 466-472.
- [28] Chen X and Oppenheim JJ. Therapy: paradoxical effects of targeting TNF signalling in the treatment of autoimmunity. *Nat Rev Rheumatol* 2016; 12: 625-626.
- [29] Atzeni F, Talotta R, Salaffi F, Cassinotti A, Varisco V, Battellino M, Ardizzone S, Pace F and Sarziputtini P. Immunogenicity and autoimmunity during anti-TNF therapy. *Autoimmun Rev* 2013; 12: 703-708.
- [30] Willrich MA, Murray DL and Snyder MR. Tumor necrosis factor inhibitors: clinical utility in autoimmune diseases. *Transl Res* 2015; 165: 270-282.
- [31] Fiore E and Vitti P. Serum TSH and risk of papillary thyroid cancer in nodular thyroid disease. *J Clin Endocrinol Metab* 2012; 97: 1134-1145.
- [32] Ott J, Promberger R, Kober F, Neuhold N, Tea M, Huber JC and Hermann M. Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case-control study in women undergoing thyroidectomy for benign goiter. *Thyroid* 2011; 21: 161-167.
- [33] Watt T, Hegedus L, Bjorner JB, Groenvold M, Bonnema SJ, Rasmussen AK and Feldt-Rasmussen U. Is thyroid autoimmunity per se a determinant of quality of life in patients with autoimmune hypothyroidism? *Eur Thyroid J* 2012; 1: 186-192.
- [34] Ding ZR, Xia LH and Yue XH. Comparison of Chinese and Western medicine therapy for children with hashimoto's thyroiditis combined hypothyroidism. *Int J Clin Exp Med* 2016; 9: 4153-4157.
- [35] Sa EH, Jin UH, Kim DS, Kang BS, Ha KT, Kim JK, Park WH and Kim CH. Herbal medicine gamgungtang down-regulates autoimmunity through induction of TH2 cytokine production by lymphocytes in experimental thyroiditis model. *J Ethnopharmacol* 2007; 109: 472-479.