The addition of ribavirin to interferon-α therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism

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Abstract

Objective: The aim of this study was to investigate whether the addition of ribavirin (RIBA) to interferon-α (IFN-α) therapy increases the risk of developing thyroid autoimmunity and/or dysfunction. Design and Methods: The study group (group A) included 72 patients undergoing treatment with IFN-α (3–6 million units three times weekly) plus RIBA (1.0–1.2 g/day) for chronic hepatitis C (CHC), as first line therapy (30 cases) or as a second therapeutic attempt after a previous ineffective IFN-α treatment (42 cases). The control group (group B) encompassed 75 age- and sex-matched patients affected by CHC, undergoing treatment with IFN-α alone as first line therapy (35 cases) or as a second therapeutic attempt (40 cases). Thyroid autoimmunity and function were retrospectively evaluated on frozen aliquots, drawn before, after 6 months, and at the end of the antiviral treatment. In patients receiving two antiviral treatments (42 cases in group A and 40 cases in group B) thyroid parameters were also assayed on serum samples drawn before and at the end of the first IFN-α therapy.

Results: Thyroid autoimmunity rate (17/72 for group A and 17/75 for group B) as well as anti-thyroglobulin antibodies (TgAb) and anti-thyroid peroxidase antibodies (TPOAb) serum levels were comparable between the two groups. Similarly, in patients undergoing two consecutive antiviral treatments (42 cases in group A and 40 cases in group B) the percentage of positivity for thyroid autoantibodies did not change significantly from the first to the second therapeutic schedules in both groups, with no significant increase of median TgAb and TPOAb levels. By the same token, all but one patient negative for thyroid autoantibodies at the end of a previous treatment with IFN-α alone were protected from the development of thyroid autoimmunity and/or dysfunction in a second course of antiviral treatment with IFN-α + RIBA. The rate of hypothyroidism (11/72 for group A) was significantly higher than that observed in group B (3/75). Similarly, in patients undergoing two consecutive antiviral treatments the percentage of hypothyroidism increased significantly from the first to the second therapeutic schedule in group A (from 4.8% to 19.0%; P < 0.05) but not in group B (from 4.7% to 7.1%; not significant). In group A, the occurrence of hypothyroidism during treatment with IFN-α + RIBA was significantly correlated with a long-term remission of CHC.

Conclusions: Our study shows that: (i) the addition of ribavirin to IFN-α therapy for CHC does not modify the thyroid autoantibody pattern but it is associated with a higher risk of hypothyroidism; (ii) the patients without thyroid autoantibodies at the end of a previous treatment with IFN-α alone are protected from the development of thyroid autoimmunity and/or dysfunction in a second course of antiviral treatment with IFN-α + RIBA; (iii) the development of hypothyroidism in patients with thyroid autoantibodies undergoing treatment with IFN-α + RIBA is significantly associated with the long-term remission of CHC.

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Introduction

Thyroid autoimmunity and dysfunction have been widely reported as side effects of interferon therapy, although with controversial data regarding the incidence, severity and physiopathological mechanism of such manifestations (1–7). In a recent paper, we have shown that the interferon-α (IFN-α)-related thyroid autoimmunity is not a homogeneous disease, but encompasses a spectrum of disease activities, probably as an expression of different underlying genotypes (8).
Until recently, IFN-α has been considered the only option available for the treatment of chronic hepatitis C (CHC), with sustained remission in about 20–25% of cases (9). In recent years, it has been established that the combination of IFN-α with ribavirin (RIBA) may increase the biochemical and virological response rates in CHC patients to about 40% (10). Currently, the combination therapy of IFN-α and RIBA is the first line treatment in naïve patients and a good therapeutic option in relapsers and non-responders (11, 12).

The therapeutic effectiveness of RIBA in CHC seems to be correlated to its immunomodulatory effects, although anti-inflammatory action has recently been demonstrated (13). In particular, RIBA seems to modulate the T helper (Th) 1 and Th2 subset balance by activating type 1 cytokine in the hepatitis C virus (HCV)-specific immune response (14, 15). This shift is critical for the control of HCV infection, since the Th1 defect is an important determining factor in the progression of the disease (16, 17). However, RIBA can also enhance the non-virus-induced immune response (18), suggesting that this drug, as a type 1-inducing agent, could trigger autoimmune phenomena in predisposed patients. Indeed, it has been demonstrated that the Th1-like immune response is an important factor for the development and maintenance of organ-specific autoimmune diseases (19). In Hashimoto’s thyroiditis, in particular, the Th1-polarized immune profile seems to be critical for the disease activity (20). However, few reports have aimed to investigate the role of RIBA on the occurrence of autoimmune disorders (21–23) and the data evaluating such therapy and thyroid autoimmune disorders are even more limited (24–26).

The aim of this study was to investigate whether the addition of RIBA to IFN-α therapy increases the risk of developing thyroid autoimmunity and/or dysfunction. Moreover, we aimed to evaluate whether the appearance of thyroid autoimmunity disease during treatment with IFN-α plus RIBA was correlated with the long-term remission of CHC.

Patients and methods

Patients

Our study group (group A) included 72 patients (25 females, 47 males; median age 47 years, range: 27–65) with serological (elevated alanine-aminotransferase activity and presence of HCV antibodies and HCV-RNA positive) and histological diagnosis of CHC, undergoing treatment with IFN-α (3–6 million units three times weekly) and RIBA (1.0–1.2 g/day) for 5–12 months at the Second University of Naples, from January 1996 to December 1999. Thirty patients received IFN-α+RIBA as the first line therapy, whereas the other 42 patients were non-responders or relapsers to a previous treatment with IFN-α alone (Fig. 1, Table 1). In the latter patients, the wash-out period between the two treatments ranged from 4 to 24 months. All patients gave written informed consent to treatment with IFN-α plus RIBA.

The control group (group B) encompassed 75 patients affected by CHC, with comparable sex (23 females, 52 male) and age (median 49 years, range: 29–64) to those of group A, admitted to our hospital in the same time period as group A. These patients refused the treatment with IFN-α plus RIBA and underwent treatment with IFN-α alone as first line therapy (35 cases) or as a second therapeutic attempt after a first ineffective IFN-monotherapy (40 cases) (Fig. 1, Table 1). The wash-out period between the two monotherapies was comparable for the two groups (Table 1). Moreover, the duration of the second treatment in group B was comparable to that of group A (Table 1).

Figure 1 shows that the study design consisted of two parts: (i) a cross-sectional study performed on 72 patients of group A and 75 patients of group B undergoing treatment with IFN-α plus RIBA and IFN-α alone respectively; (ii) a longitudinal study performed on 42 of 72 patients of group A and 40 of 75 patients of group B who received two consecutive antiviral treatments.

**Figure 1** Study flow chart. The cross-sectional study was performed between 72 patients in group A (Gr. A) and 75 patients in group B (Gr. B). The longitudinal study was performed between 42 of the 72 patients in group A and 40 of the 75 patients in group B.
Long-term response to antiviral therapy was defined as the normalization of serum alanine-aminotransferase and the disappearance of serum HCV-RNA during therapy and for at least 12 months after the end of treatment. Thyroid autoimmunity (serum anti-thyroglobulin antibodies, TgAb; anti-thyroid peroxidase antibodies, TPOAb; thyroid stimulating hormone-receptor antibodies, TRAb) and function (serum free thyroxine (FT 4), free tri-iodothyronine (FT 3), and thyrotropin (TSH)) were retrospectively evaluated on frozen aliquots drawn before, after 6 months, and at the end of the antiviral treatment (IFN-α + RIBA treatment). All of the patients were euthyroid and negative for thyroid autoantibodies before the first treatment. Patients with thyroid diseases and to respond to antiviral therapy. A thyroid autoantibody was defined as Abs+. Overt hypothyroidism was defined by serum TSH values above the normal range, serum FT 4 below the normal range and serum FT 3 in the normal range. Patients with subclinical hypothyroidism were defined by serum TSH values above the normal range, serum FT 4 and FT 3 in the normal range. Patients with overt hypothyroidism were not treated with L-thyroxine, but they were followed throughout the study.

**Methods**

Serum FT 4 and FT 3 were assayed by double antibody RIA (Technogenetics, Milan, Italy), and serum TSH was assayed by an immunoradiometric method (DIA-Sorin, Saluggia, Italy). Samples were assayed in duplicate for each hormone. The detection limit of the assays in SI, the intra- and interassay variations expressed as coefficients of variation were respectively: 1.2 pmol/l, 2.9% and 4.7% for FT 4; 1.3 pmol/l, 3.0% and 5.7% for FT 4; 0.05 mU/l, 3.1% and 4.2% for TSH. In our laboratory, normal values were 3.8–7.7 pmol/l for FT 4, 9.0–23.1 pmol/l for FT 3 and 0.3–3.5 mU/l for TSH. TgAb (negative <100 U/ml) were measured using the immunoradiometric assay (BioChem ImmunoSystem, Bologna, Italy) with intra-assay and interassay coefficients of variation and detection limit of 3.9%, 6.9% and 5.0 U/ml respectively. TPOAb (negative <10 U/ml) were measured using a RIA kit (DIA-Sorin) with intra-assay and interassay coefficients of variation and detection limit of 2.5%, 6.6% and 0.7 U/ml respectively. TRAb (negative <10 U/ml) were tested by radioreceptor assay (RRA) (Henning, Berlin, Germany).

**Statistical analysis**

Results are expressed as median and ranges. Before performing the data analysis, the nQuery test was used to predict the adequacy of sample size in cross-sectional study. Un-paired data were compared by Mann–Whitney test and frequencies between different groups were compared by χ2 and Fisher exact tests. Paired data and repeated frequencies (in those patients who received two consecutive antiviral treatments: 42 cases in group A and 40 cases in group B) were compared by Wilcoxon and McNemar tests respectively. A logistic regression model was used for the analysis of probability to develop thyroid diseases and to respond to antiviral therapy. A P value <0.05 was considered significant.

**Results**

**Cross-sectional study**

At the study entry, 7 of 72 patients in group A (9.7%) and 8 of 75 patients in group B (10.7%) were Abs + (χ2 = 0.03; P = 1.0). After IFN-α + RIBA treatment,
thyroid autoimmunity was found in 17/72 patients (23.6%) in group A and a comparable rate was observed in group B (17/75 patients; 22.7%) after treatment with IFN-α alone ($\chi^2 = 0.02; P = 1.0$). No statistical difference in TgAb (1150 U/ml, range: 430–7890 in group A vs 879 U/ml, range: 390–14 000 in group B; not significant (ns)) and TPOAb (760 U/ml, range: 23–987 in group A vs 854 U/ml, range: 110–2400 in group B; ns) levels was observed between the two groups. None of the patients developed TRAb throughout the study.

The rate of hypothyroidism in group A (11/72) was significantly higher than that observed in group B (3/75) ($\chi^2 = 5.4; P = 0.03$). Table 2 shows the individual biochemical data of the 14 patients who developed hypothyroidism. Five patients from group A showed an overt hypothyroidism, while the remaining 9 patients (6 from group A and 3 from group B) developed a subclinical hypothyroidism (Table 2). Hypothyroidism was accompanied in all cases by the presence of thyroid autoantibodies (Table 2). L-Thyroxine treatment was accompanied in all cases by the presence of thyroid autoantibodies (Table 2). L-T4 therapy. Those values in bold are outside the normal ranges.

### Table 2

<table>
<thead>
<tr>
<th>Group/Sex</th>
<th>Previous IFN-α treatment</th>
<th>Start of treatment</th>
<th>At the end of treatment (or before L-T4 therapy)</th>
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<td>Yes</td>
<td>TSH (mU/l)</td>
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</tr>
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<td>12.7</td>
</tr>
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</table>

Normal range values for FT4, FT3 and TSH are: 9.0–23.1 pmol/l for FT4; 3.8–7.7 pmol/l for FT3; 0.3–3.5 mU/l for TSH. TgAb negative = < 100 U/ml and TPOAb negative = < 10 U/ml.

The patients treated with IFN-α+RIBA (group A) showed higher long-term remission of CHC than patients treated with IFN-α alone (group B) (26.4% vs 10.7%; $\chi^2 = 6.1; P = 0.02$). In group A, the long-term remission of CHC occurred in 7 of 17 patients who were Abs + (41.2%) and in 12 of 55 patients who were Abs – (18.9%); such percentages were not significantly different ($\chi^2 = 2.5; P = 0.1$). Taking into account the patients of group A who developed hypothyroidism (11 cases), a long-term remission of chronic infection was demonstrated in 7 of them (63.6%); such percentage was significantly higher than that (19.7%) observed in patients who remained euthyroid throughout the antiviral treatment ($\chi^2 = 9.3; P = 0.006$).

In group B, the long-term remission of CHC occurred in 1 of 3 patients (33.3%) who showed hypothyroidism in the course of IFN-treatment and in 7 of 72 patients (9.7%) who remained euthyroid; these percentages were not significantly different ($\chi^2 = 1.7; P = 0.3$).

Logistic regression analysis demonstrated that the long-term remission of CHC was significantly correlated with the occurrence of hypothyroidism during the treatment with IFN-α + RIBA (odds ratio: 17.0, C.I. 95% 1.8–28.4), but not with thyroid autoimmunity (odds ratio: 2.5, C.I. 95% 0.8–8.0). In group B, the long-term remission of CHC in the course of treatment with IFN-α alone was not correlated to either thyroid autoimmunity (odds ratio: 1.4, C.I. 95% 0.3–61.0) or hypothyroidism (odds ratio: 4.6, C.I. 95% 0.4–57.9).

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Longitudinal study

In patients who received two consecutive therapeutic schedules (42 cases in group A and 40 cases in group B), no significant increase in thyroid autoimmunity rate was observed from the first to the second treatment (Table 3), without significant increase in median TgAb (group A: from 1272 U/ml, range: 795–6679, to 1303 U/ml, range: 445–7890; group B: from 712 U/ml, range: 160–15 000, to 1160 U/ml, range: 200–1987) and TPOAb levels (group A: from 494 U/ml, range: 134–7900; group B: from 667 U/ml, range: 130–1987, to 971 U/ml, range: 423–2100; ns in both groups). All but one patient negative for thyroid autoantibodies at the end of the first treatment remained so during the subsequent treatment in both therapeutic groups.

In patients of group A the percentage of hypothyroidism increased significantly from the first (IFN-α alone) to the second (IFN-α + RIBA) therapeutic schedule (from 4.8% to 19.0%; P = 0.03) (Table 3), with a further impairment of thyroid function in those patients with pre-existing subclinical hypothyroidism (Table 2). On the other hand, in group B the second treatment with IFN-α alone induced neither significant modification of the hypothyroidism rate (4.7% in the first and 7.1% in the second monotherapy; P = 0.5) (Table 3), nor a significant failure of thyroid function in patients with pre-existing subclinical hypothyroidism (Table 2).

Discussion

Our longitudinal study shows that the percentage of thyroid dysfunction is significantly higher in patients undergoing therapy with IFN-α plus RIBA than that observed in the course of treatment with IFN-α alone, without significant modification of serum autoantibody levels.

The combination of RIBA with IFN-α did not significantly modify the percentage of thyroid autoimmunity rate nor thyroid autoantibody levels with respect to IFN-α treatment alone. This finding has been confirmed by the within-subject controlled analysis performed in patients who had received two subsequent antiviral treatments. In fact, the patients without thyroid autoantibodies at the end of the first IFN-α treatment remained so also in the course of the second treatment with either IFN-α alone or IFN-α + RIBA. This finding is in agreement with the observation that a genetic predisposition is important in the development of the cytokine-induced autoimmunity (27, 28). Moreover, it has been demonstrated recently in a long-term follow-up study that the negativity for thyroid autoantibodies after IFN treatment is a protective factor for developing thyroid autoimmunity and/or dysfunction in the following years (8).
Hypothyroidism is a frequent effect of IFN-α treatment (29). Some authors have reported the occurrence of a biphasic thyroiditis characterized by a transient phase of thyrotoxicosis followed by hypothyroidism (29). Such clinical presentation has also been described in patients treated with IFN-α + RIBA (24, 30). In our study, however, none of patients showed thyrotoxicosis, whereas hypothyroidism was the only described thyroid dysfunction. Probably the wide interval between the retrospective determinations of thyroid status did not allow us to evaluate whether the hypothyroidism was preceded or not by a transient phase of thyrotoxicosis. Indeed, both hypothyroidism and thyrotoxicosis could be considered as different clinical expressions of a destructive process in thyroid parenchyma. In this view, the higher percentage of hypothyroidism observed in patients treated with IFN-α + RIBA than that found in patients treated with IFN-α alone could suggest the occurrence of a high degree of thyroid damage during the combined treatment.

The immunological mechanisms responsible for the high risk of hypothyroidism in the course of treatment with IFN-α + RIBA cannot be clarified in the present work. However, we believe that the immunomodulatory effects of RIBA may, at least in part, explain our clinical findings. In fact, RIBA is a guanosine analog that plays important immunological effects in vivo, by a differential modulation of Th1- and Th2-like responses, with a specific shift favouring the Th1-like activity (14, 15). In thyroid autoimmune disease, while the Th2 lymphocytes regulate the autoantibody production, the Th1 subsets have limited B cell help capacity and are mediators of tissue damage (19, 20). In our cases, RIBA could induce hypothyroidism by Th1-dependent activation of CD8+ T lymphocytes which induce cellular destruction predominantly by the perforin pathway (31). By the same token, IFN-α favors such a mechanism by enhancing the expression of perforin molecules in peripheral lymphocytes, as recently demonstrated in an in vitro model (32). The thyroid cell damage in RIBA-induced hypothyroidism could also be due to a complement-mediated injury. In fact, the activation of Th1 lymphocyte subsets by RIBA leads to the selective production of immunoglobulin (Ig) G2a subclass autoantibodies (33, 34), which have shown the highest functional affinity for complement (35). An additional mechanism responsible for the RIBA-induced hypothyroidism in our patients could be the apoptosis of follicular cells. Indeed, RIBA as inhibitor of inosine 5’-phosphate dehydrogenase has been shown to regulate cellular differentiation, proliferation, and apoptosis in in vitro models (36, 37).

In our experience, the remission of CHC seems to be correlated with the appearance of hypothyroidism in patients treated with IFN-α+RIBA but not in patients treated with IFN-α alone, as previously reported (38). Nevertheless, it seems difficult to propose RIBA-induced occurrence of hypothyroidism as a reliable clinical tool for predicting the long-term response to antiviral therapy because most patients with remission of CHC have shown neither thyroid autoimmunity nor thyroid dysfunction. However, the Th1-like mechanism shared by both autoimmune hypothyroidism (19, 20) and viral clearance (16), may contribute to this finding.

In conclusion, our study shows that: (i) the addition of ribavirin to IFN-α therapy for CHC does not modify the thyroid autoantibody pattern but exposes the patients to a higher risk of hypothyroidism, probably as a consequence of different effects of ribavirin on Th1- and Th2-like immune responses; (ii) the patients without thyroid autoantibodies at the end of a previous treatment with IFN-α alone are protected against the development of thyroid autoimmunity and/or dysfunction in the course of a second antiviral treatment with IFN-α+RIBA; (iii) the development of hypothyroidism in patients with thyroid autoantibodies in the course of treatment with IFN-α+RIBA is significantly associated with the long-term remission of CHC. Such correlation is lacking in patients undergoing treatment with IFN-α alone.

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