Graves’ Disease: Thyroid Function and Immunologic Activity

A. A. R. Gossage, J. C. W. Crawley, S. Copping, D. Hinge, and R. L. Himsworth

Clinical Research Centre, Harrow, Middlesex, HA1 3UJ, UK

Patients with Graves’ disease were studied for two years during and after a twelve-month course of treatment. Disease activity was determined by repeated measurements of thyroidal uptake of \(^{99m}\text{Tc}\) pertechnetate during tri-iodothyronine administration. These in-vivo measurements of thyroid stimulation were compared with the results of in-vitro assays of Graves, immunoglobulin (TSH binding inhibitory activity—TBIA). There was no correlation between the thyroid uptake and TBIA on diagnosis. Pertechnetate uptake and TBIA both declined during the twelve months of antithyroid therapy. TBIA was detectable in sera from 19 of the 27 patients at diagnosis; in 11 of these 19 patients there was a good correlation (p < 0.05) throughout the course of their disease between the laboratory assay of the Graves, immunoglobulin and the thyroid uptake. Probability of recurrence can be assessed by sustained remission of Graves’ disease after treatment cannot be predicted from either measurement alone or in combination.


It is now generally accepted that the hyperthyroidism of Graves’ disease is due to the interaction of a specific immunoglobulin G (IgG) with the thyroid cell membrane at the thyrotrophin receptor. This interaction provokes the unregulated overactivity that is characteristic of the thyroid in Graves’ disease, which cannot be suppressed, as in normal subjects, by the administration of tri-iodothyronine (T\(_3\)).

The thyroid-interactive IgGs of Graves’ disease (henceforth referred to as Graves, immunoglobulins) are believed to be of variable effectiveness as thyroid stimulators. The correlation between in-vitro measurements of these IgGs and the clinical state of the patient has generally been poor. Although some workers have found a correlation between such measurements and the uptake of iodine by the thyroid (1–3), others have not (4,5). If the biological effectiveness of the Graves immunoglobulins varies between patients, such variability might confound any relation between in-vivo and in-vitro estimates of disease activity. We therefore undertook a prospective trial, making sequential observations on a group of patients of thyroidal uptake of pertechnetate (\(^{99m}\text{TcO}_4^-\)) and the ability of their IgG to inhibit the binding of TSH to thyroid cell membranes in vitro (a measure of the content of specific Graves immunoglobulin).

PATIENTS AND METHODS

Thirty-three patients were recruited. The only criteria for inclusion were that antithyroid drugs were the treatment of first choice, and that the patient was willing to take part in the study and able to give informed consent for an investigation that had been approved by the Hospital Ethical Committee. All were under the age of 45 yr. The plan of management and the timing of the various tests are shown in Fig. 1. The antithyroid drugs were given in full dosage throughout the twelve months after diagnosis to prevent any production of thyroxine. Carbimazole (CMZ), 15 mg 8-hourly, was the preferred regimen, and, if a reaction to CMZ occurred, then propylthiouracil (PTU) was given instead, 200 mg three
times a day. When the concentration of thyroxine in the serum had fallen into the low-normal range, triiodothyronine was added, 20 µg three times daily, or 40 µg in the morning and 20 µg in two further doses at intervals of 8 hr, usually at 4–6 wk after beginning treatment. The suppression of pituitary production of TSH was checked by tests with TSH-releasing hormone (TRH). The patients were studied for two years; antithyroid drugs were given for the first twelve months and T3 was continued until 18 mo. Serum samples obtained at each attendance were stored at −30°C until all were assayed together at the end of the study.

Thyroid size was estimated in grams by palpation, always by the same observer. Thyroid hormone levels were measured by radioimmunoassay.

The assays of TSH-binding inhibitory activity (TBIA).

Our method for the assay of TBIA of IgG has been fully described (6) and is based upon that of Smith and Hall (7). Samples from an individual patient were all assayed at the same time at the end of the two-year protocol within a single assay and with the same thyroid-membrane preparation. The IgGs were isolated from the other serum proteins and added in a fixed concentration.

The TBIA value was derived from the calculation:

\[ \frac{I-125 \text{ TSH bound in presence IgG}}{I-125 \text{ TSH bound in absence IgG}} \times 100 \]

Normal IgG has a variable effect in this assay. Samples of normal IgG were therefore included in every assay and the maximum interference was subtracted from the results of the calculation. Thus any value greater than 0 indicates definite Graves' IgG inhibitory activity; values below 0 may have biological significance but are submerged into the range of effects caused by normal IgG.

Thyroid uptake measurements. The thyroid uptake of pertechnetate (99mTcO4−) was measured at 20 min using a hybrid scanner (8). This instrument consisted of two opposing line detectors, one above and one below a couch that moved the patient longitudinally, at right angles to the axis of the detectors. Data were stored on magnetic tape during a scan, and a computer was used to produce a line-printer image of the patient's body. Areas could be selected on the image and the counts within those areas expressed as a percentage of a known activity in a phantom or as a percentage of the entire body. The computer program also corrected for the decay of the Tc-99m.

Correction for background radioactivity in the neck was based upon measurement of a similar area of the thigh (9). The validity of this correction when used with our equipment was confirmed by giving two volunteers Tc-99m-labeled serum albumin and perchlorate intravenously and showing that the correction for background activity in the neck could be made by subtraction of twice the activity over the thigh. In a patient being studied, the areas selected for the thyroid and thigh in the first measurement were used for all subsequent measurements.

Because each patient acted as his or her own control, it was not considered necessary or ethical to establish systematically a range of normal values for pertechnetate uptake on our equipment. Measurements on normal subjects made for other purposes, however, fell within the range (0.4–3.0 percent of the dose) determined by others who scanned the thyroid with a small line detector (10).

RESULTS

Thirty-three patients entered the study. All had clinically obvious Graves' disease and at diagnosis the serum T4 ranged from 150 to 400 nmole/l (mean 241 nmole/l ± 68 s.d.). Six were withdrawn for various reasons (three failed to take their drugs, one developed hepatitis, one became pregnant, and one moved away). At the end of two years, 16 of the 27 patients who had completed the study remained in remission (Group 1) whilst the remaining 11 had relapsed (Group 2). In retrospect these two groups could not be distinguished on presenting symptoms, signs, or biochemistry.

The results of repeat measurements of thyroidal pertechnetate uptake over the two-year period are shown in Fig. 2. Every patient had a raised thyroidal uptake of pertechnetate on presentation, range 4.2 to 23.6 percent of dose administered [mean (9.2 ± 4.2) percent]. All patients showed a decrease in thyroid uptake during their year of antithyroid drug therapy, but there were wide variations in the rate of decline. There was no significant difference at 0 and 1 mo between the group that relapsed after treatment and the group that remained in remission. There were significant differences (Fig. 2) between the two groups of patients (Wilcoxon's sum of ranks test).
at 3 mo (p <0.05) and at 6, 9, and 12 mo (p <0.01). In only one patient did the uptake decline to less than 1%, and this did not occur until the fifteenth month after diagnosis. Normal subjects given T3 in this fashion took up less than 0.5% of a dose of pertechnetate into the thyroid. No patient in this series, therefore, can be considered to have had a thyroid that was normally suppressible by T3 at the time of antithyroid drug withdrawal.

There was no significant correlation between the initial thyroid uptake of pertechnetate and the thyroid size at the time of presentation (p <0.1). A correlation was found between the initial value of pertechnetate and the initial values of serum T4 (r = 0.45, p <0.01), and the serum T3 (r = 0.4, p <0.02).

At the end of the first year it would be expected that failure of T3 to suppress thyroidal uptake of pertechnetate would be associated with a similar failure of T3 to suppress T4 production after the withdrawal of antithyroid drugs. Thyroxine production was suppressed completely or partially by continued administration of T3 from 12 to 18 mo in Group 1 patients but not those in Group 2. In the 27 patients who completed the study there was a significant correlation between the uptake of pertechnetate at 12 mo and the serum T4 concentration at 13 mo (r = 0.67, p <0.01).

Nineteen of the 27 patients had a positive TBIA at the time of presentation: 10 of the 16 in Group 1, and 9 of the 11 in Group 2. There was no correlation between the initial values of TBIA and pertechnetate uptake (r = 0.32, p <0.1). During the period of treatment with antithyroid drugs, the TBIA declined into the normal range in all patients in Group 1, and this fall was accompanied by a decrease in thyroidal uptake of pertechnetate. The TBIA values for the patients in Group 1 remained thereafter within the normal range. In five patients in Group 2 the TBIA and pertechnetate fell in parallel into the normal range and then rose together to abnormal levels at the time of recurrence of hyperthyroidism. The results from one of these latter patients are shown in Fig. 3. In four patients of Group 2 with demonstrable TBIA at presentation, a divergence developed during the course of the study between the two indices of activity of Graves' disease: a fall in thyroidal uptake of pertechnetate occurred, unaccompanied by any change in TBIA. Two patients in Group 2 did not have clearly detectable TBIA on diagnosis; nevertheless when their disease recurred after cessation of treatment, the results of the assay became positive.

In eleven of the 19 patients with positive TBIA on diagnosis, and in whom there was sufficient range of change, the correlation between the serial values of pertechnetate uptake and TBIA in each individual patient was good (p <0.05). In four patients there was insufficient range of change of TBIA to warrant any conclusion.

DISCUSSION

We have demonstrated in the majority of our patients with Graves' disease that, in any subject followed with serial tests over two years, there is a close and persistent relation between measurements of abnormal stimulation of the thyroid, as judged by the uptake of pertechnetate and the results of an in-vitro assay for the specific Graves' immunoglobulins. This finding reaffirms the significance of measurements of thyroid activity and
suppressibility in the assessment of Graves' disease, and, further, relates them directly to the underlying immunological disorder.

Although all the patients had Graves' disease, and all had an elevated uptake of pertechnetate by the thyroid at diagnosis, not all had detectable amounts of Graves' immunoglobulins in their sera by the technique used. Our assay detects the presence of Graves' immunoglobulins in about 70% of new cases and gives results comparable with those of others using the same method (4,11–15). This assay measures the quantity of immunoglobulin that can interact with the TSH receptor; it does not measure activation of TSH receptor mechanism. Graves' immunoglobulins are heterogeneous and of varied biological effectiveness. It is likely, therefore, that patients with Graves' disease but no detectable Graves' immunoglobulin by this method are producing a small quantity of biologically potent immunoglobulins. This inference is supported by the higher proportion of patients who have Graves' immunoglobulins at diagnosis as measured by more sensitive assays using indices of receptor activation (13,16–18). The heterogeneous nature of Graves, immunoglobulins is indicated by the lack of correlation between the uptake of pertechnetate and TBIA measurements in our patients at diagnosis. This finding is in general agreement with the work of others (4,5) although some have found a rough correlation (1–3). Nevertheless, we have shown, for the first time, that in any individual patient followed for two years there is more often than not a high degree of correlation between the two measurements. This suggests that the biological character of the immunoglobulins is consistent but their concentration in the serum varies with the clinical course of the disease.

The thyroid uptake of pertechnetate declined in all patients during treatment with antithyroid drugs. This decline continued in the patients of Group 1 during the succeeding 6 mo, when T₃ alone was being given (Fig. 2). The thyroidal uptakes of the patients in Group 2 also declined during treatment of hyperthyroidism for the first twelve months, although on average the values were persistently higher than those in Group 1. On relapse the pertechnetate uptake rose in all patients in Group 2. The earliest relapse occurred one month after antithyroid drugs were stopped; a further seven patients relapsed 3 to 6 mo after withdrawal of drugs but whilst still taking T₃; and three patients relapsed during the last 6 mo of the study when they were off treatment. Nevertheless, there were in Group 2 some patients whose pertechnetate uptake was within the normal range at 21 mo; it might have been anticipated that these patients would remain in remission. However, prediction of sustained remission by tests of thyroid uptake or TBIA is not reliable because resurgence of hyperthyroidism may follow a course of medical treatment with normalization of both measures of disease activity.

Our main purpose was to study the relationship between in-vivo determination of Graves' disease activity by pertechnetate uptake and the in-vitro measurements of Graves' immunoglobulins. We have found that in most individual patients followed serially there is a good correlation, although this is not true when data from different patients are pooled.

The predictive values of tests of thyroid uptake have been variously reported. Thus Alexander and colleagues (19) reported that good predictions could be made of the likelihood of remission or recurrence of hyperthyroidism when the thyroid suppressibility was tested after 6 months of antithyroid drug therapy, but they later reported that the predictions made at that time were not sufficiently reliable to be useful (20,21). Goolden et al. (22) did not find assessments at 6 mo to be helpful, but nevertheless found that the majority of patients who were going to remain in remission could be identified before a full year of antithyroid therapy. Alexander and colleagues (21) recommend that if the thyroidal uptake of 131I was still not suppressible by T₃ after 2 yr of antithyroid drug therapy, one should recommend surgical treatment or radioiodine therapy. Others (23) have suggested that this decision can be made after one year of antithyroid drug therapy. We have confirmed that a persistently elevated uptake of Tc-99m at the end of a period of treatment with antithyroid drugs indicates a strong probability of resurgence of hyperthyroidism. This is also true if Graves' IgG can still be demonstrated by in-vitro tests. Prediction of sustained remission, however, is much less reliable, and values for TBIA and Tc-99m uptake within the normal range do not preclude a subsequent recurrence of hyperthyroidism.

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REFERENCES