Monoclonal Immunoradiometric Assay of Calcitonin Improves Investigation of Familial Medullary Thyroid Carcinoma

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Calcitonin (CT) assay is essential for recognizing medullary thyroid carcinoma (MTC), particularly occcluding familial MTC. In previous radioimmunassays of calcitonin, polyclonal antibodies were used. Here we evaluate a new two-site immunoradiometric assay (IRMA) of calcitonin based on use of monoclonal antibodies. We assayed samples from healthy subjects, patients with renal failure, and subjects from families affected by MTC. Basal values for healthy subjects were all <10 ng/L. Renal failure is associated with increased basal CT. The CT peak under pentagastrin stimulation in healthy patients was <30 ng/L. In familial screening, basal values >10 ng/L or peak values >30 ng/L correspond to subjects with histologically confirmed MCT or micro-MCT. Polyclonal RIA performed in the same subjects failed to detect the moderate increase of CT that IRMA demonstrated. Preliminary results indicate that this new method may allow earlier detection of CT increase and thus improved diagnosis of MCT, particularly in familial screening. Monitoring surgical patients could also be improved by this new assay.

Additional Keyphrases: screening • cancer • radioimmunoassay • heritable disorders • pentagastrin stimulation test • early detection

Medullary carcinoma of the thyroid (MCT), as described by Hazard et al. (1), is transmitted by heredity in at least 25% of the cases. Early diagnosis and treatment of hereditary cases relies on assay of calcitonin (CT) after stimulation with pentagastrin. Patients with MCT have been detected because of an increased basal value or, at least, by a significant increase in CT concentrations in serum after pentagastrin injection.

Until now, in CT assays polyclonal antibodies have been used (2–4). Motté et al. (5) recently described an immunoradiometric assay (IRMA) involving use of two monoclonal antibodies. The aim of the present study was to evaluate this new method and to attempt to answer two questions: can this IRMA recognize microscopic lesions of MCT better than the usual radioimmunoassay (RIA), and what are the criteria for interpreting results of the pentagastrin test with IRMA?

Materials and Methods

Assays

Immunoreactive CT was measured simultaneously for every patient with the monoclonal antibody IRMA and with one of the three RIAs described below, in all of which...
polyclonal antibodies were used.

RIA's. The methodology of the three RIAs is similar. Synthetic CT labeled with $^{125}\text{I}$ is recognized by the antibody, then the free and bound fractions of $^{125}\text{I}$-labeled CT are separated by using dextran-coated charcoal or a second antibody. The specific antibodies used in two of the methods are obtained from sheep immunized with synthetic monomeric CT (Ab M732 and Ab 600017); the third method is a commercial kit method (CIS International, Gif sur Yvette, France) in which rabbit antibodies (Ab3) are used. All three antibodies are directed mainly against determinants in the middle part of the molecule. For all three assays the lowest detectable concentration of CT is about 25 ng/L.

IRMA. The assay of Motte et al. (5) is now available commercially (ELSA-hCT; CIS Bioindustries, Gif sur Yvette, France). In it are used two monoclonal antibodies, directed against different determinants. The second antibody is labeled with $^{125}\text{I}$. A single incubation of both antibodies for 24 h is carried out in the presence of plasma heated to 56 °C. The antibodies recognize the 11–17 and 24–32 amino acid regions of the monomeric CT. Only the intact monomeric form of CT may be determined with this method. The lowest detectable concentration is 2.5 ng/L.

Subjects

Healthy subjects. Basal concentrations of CT were determined in 83 control subjects, ages 7 to 60 years. A pentagastrin stimulation test (6) was performed in 18 healthy volunteers, ages 20 to 35 years; they received a slow intravenous injection of 0.5 μg of pentagastrin ("Peptavlon"; Imperial Chemical Industry Pharma, Enghien, France) per kilogram body weight during 3 min, and CT was assayed in their blood sampled 5 min before and 0, 3, 5, and 10 min after this injection. For RIA methods, the response is expressed as the ratio of the maximum peak to the basal value; the response is considered abnormal when this ratio exceeds 2.5.

Patients with renal failure. The basal CT concentrations were determined in 13 chronic hemodialyzed patients without known thyroid disease.

MCT patients (with clinical primitive or recurrent tumor). The CT basal concentration was determined in seven patients presenting with histologically confirmed medullary carcinoma, appearing clinically as one or several thyroid nodules. The follow-up of 21 patients who had already had surgery for MCT revealed a marked increase of CT in the first two years after surgery, indicating recurrence of the disease.

Family study. We also assayed samples from six children from one family who underwent a pentagastrin stimulation test. The mother had a histologically confirmed MCT that was recognized because of a clinically detectable thyroid nodule.

Results

Basal Values

Healthy subjects. The basal values for CT in 95% of the normal subjects were <250 ng/L by the RIAs with Ab 60017 and M732, and <160 ng/L by the RIA with Ab3. With the IRMA, 83% of healthy subjects had basal concentrations ≤5 ng/L and the 97.5th percentile was about 7.1 ng/L, and all subjects had a basal value ≤10 ng/L.

Patients with renal failure. Eight of the chronically hemodialyzed patients had CT concentrations between 10 and 52 ng/L. The five others had values <10 ng/L.

MCT patients with clinically palpable tumors. In these patients the basal values were always increased, whatever the assay (RIA or IRMA), although RIA usually gave higher values than IRMA.

Pentagastrin Stimulation Tests

Healthy subjects. With the IRMA, nine of 18 subjects showed no increase of calcitonin after pentagastrin administration. The other nine subjects gave a response >5 ng/L: in two the peak was <10 ng/L, three reached 10 ng/L, and the others had values of 11, 16, 21, and 30 ng/L, respectively. However, the 30 ng/L peak was observed in the fifth minute in only one subject, who presented an intense vagal upset during the pentagastrin injection.

With the classical RIA methods, 17 subjects showed no response or had a peak/basal CT ratio of <2.5. For a single case this ratio was 3.2, but that subject had no thyroid disorder apparent either clinically or by echography, nor did he have any known hereditary thyroid disease.

Family study. With the RIA method, all six basal CT values were within the normal range (<250 ng/L), but three response ratios were abnormal (>2.5) (Table 1). With the IRMA method, only one child (no. 3) had a high basal value, 33 ng/L. Under pentagastrin stimulation, the response was high for four of five children. In these five children, who underwent surgery, four had medullary microscopic carcinomas (not clinically detectable) of the thyroid and one had C-cell hyperplasia.

Discussion

This new calcitonin assay is, to our knowledge, the first and only IRMA in which two monoclonal antibodies directed towards a small (32-residue) peptide are used. Only the monomer form of calcitonin is measured by this method, whereas in currently used RIA methods, which involve polyclonal antibodies, several forms of CT in the circulation are recognized.

In control subjects, the basal values obtained with IRMA are <10 ng/L. The extrathyroid pathological state must be thoroughly known before an attempt is made to interpret the result for CT as measured by this IRMA. We have found high basal values, between 12 and 52 ng/L, in dialyzed renal-failure patients with no known thyroid disease.

Patients with MCT, shown clinically by at least one thyroid tumor and confirmed by histological examination, are easily recognized by the simple measurement of the basal calcitonin concentration by IRMA, as is also the case with RIA techniques.

The most important contribution of the present IRMA is to

| Table 1. Family-Study Values for CT by RIA and by IRMA (Six Subjects) |
|-------------------------|-----------------|-----------------|-----------------|-----------------|
| CT basal and peak values, ng/L | RIA | IRMA | RIA | IRMA |
| Age, y | 14 | 10, 40 | 14 | 10, 40 |
| 15 | 87, 245 | 6, 30 | 15 | 87, 245 | 6, 30 |
| 19 | 245, 700 | 33, 347 | 19 | 245, 700 | 33, 347 |
| 21 | 50, 250 | 5 | 21 | 50, 250 | 5 |
| 23 | 150, 150 | 5, 36 | 23 | 150, 150 | 5, 36 |
| 17 | 150, 150 | 2, 4 | 17 | 150, 150 | 2, 4 |
| Histology | Micro MCT* | 10.5 | MCT* | Micro MCT* |
| | | | | C-cell hyperplasia |

Histologically confirmed microscopic (≥2 mm; ≤7 mm) medullary thyroid carcinoma.
increase the sensitivity of the pentagastrin test, especially for microscopic lesions. Half of the normal subjects have no response measurable by IRMA; of the nine subjects who did respond, seven had a response between 10 and 30 ng/L. The wide variation in response as expressed by the peak value/basal value ratio does not allow the easy use of this ratio for interpreting results of the pentagastrin test with IRMA. In our study, this ratio varied between 1 and 5.

We conclude that our assay of CT allows better identification of microscopic carcinomas in cases of hereditary MCT. The basal values may already be increased >10 ng/L but, more especially, the pentagastrin test always gave a response peak value exceeding 30 ng/L as measured with the IRMA, whereas the calcitonin determination with RIA and polyclonal antibodies revealed an abnormal response to the pentagastrin test in only half of the patients. With the caveat that chronic renal failure must first be excluded, we believe that patients whose basal calcitonin exceeds 10 ng/L and (or) those whose response to the pentagastrin test gives a peak value ≥30 ng/L when this IRMA is used must be considered as very probably having medullary carcinoma of the thyroid. Of course, these data must be validated with studies involving a larger number of cases.

References