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PATHOPHYSIOLOGIC EFFECTS OF STABLE IODINE USED AS A THYROIDAL BLOCKING AGENT TO REDUCE THYROID RADIATION EXPOSURE

Progress Report

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DISCLAIMER

ABSTRACT

In an effort to determine whether iodide administration in man can induce injury to thyroid follicular cells similar to that demonstrated in lower animals, this study measures thyroglobulin, thyroxine and triiodothyronine and thyroid autoantibodies in the serum before and after administration of iodine in a variety of patients.

In the last eight months primary efforts have been devoted to the development of a high specificity and sensitivity radioimmunoassay of thyroglobulin and its preliminary clinical application. Human thyroglobulin obtained from operative specimens was purified and its identity and purity established. Rabbits were injected with thyroglobulin for 30 weeks with the appearance of significant titres of antibodies within 6 weeks. The antibody developed was examined for immunological purity and used in a radioimmunoassay system with thyroglobulin labeled in this Laboratory with ^{125}I.

An effective assay was developed and is being used in preliminary studies. The normal serum thyroglobulin range was established in 30 volunteers with a mean of 14.3 ng/ml $\pm$ 5.07. Preliminary patient studies in hyperthyroid patients have demonstrated a tripling of already elevated thyroglobulin levels to a level 60 times normal by 24 hours after the oral administration of 250mg of iodide. Bloods have been collected following administration of various iodine containing X-ray contrast dyes but have not as yet been assayed.

PROGRESS REPORT

Introduction

The present study was undertaken to determine whether the administration of iodine to man can induce injury to thyroid follicular cells, and to define the factors that relate to the initiation and occurrence of this lesion.

The basic assumption to be tested is that iodine ingestion might produce damage to thyroid follicular epithelium and spill thyroglobulin into the serum. There it could serve as an antigen with probable subsequent appearance of thyroid autoantibodies and the development of the pathologic lesion of Hashimoto's thyroiditis, resulting in hypothyroidism as well as other thyroid disorders. The course of autoimmune processes is often relatively slow and the possible anticipated deleterious effect might not occur for prolonged periods following the precipitating event.

In an attempt to identify a susceptible subgroup of the population, it was proposed to study the incidence of thyroglobulin spillage into the serum immediately following iodine administration in patients who might have a small iodine pool such as hyperthyroid patients as well as a larger group of patients receiving iodine administration for a variety of clinical purposes.

Results: The major efforts of the investigators in the last 9 months have been directed to the development of the primary indicator for the presence of thyroglobulin in the blood, a radioimmunoassay for small amounts of thyroglobulin. These studies have proved to be somewhat more formidable than anticipated and although the development of the basic assay is now complete and has proved to be most effective, the requirements for high specificity and sensitivity have required moremanipulations than anticipated. Further work on the assay is now under way to significantly improve sensitivity. Because of the desirability of performing all sequential determinations in a single patient in the same assay, although numbers of initial test sera have been banked in the freezer, data is currently available only in a more limited number of patients.

These efforts to date including both the development of the immunoassay, its validation as well as a collection of clinical

samples have required approximately 5-10% of the efforts of the principal investigators.

Thyroglobulin Immunoassay Procedure

The thyroglobulin radioimmunoassay was adapted in the Laboratory from methods proposed by VanHerle (J.Clin. Inves. 52:1320, 1973). The work began with the collection of a fresh specimen of thyroid tissue from a patient with a nontoxic goiter. From this thyroid tissue pure thyroglobulin was isolated in the following manner: the tissue was ground and filtered and the suspension was passed through a Sephedex G-200 column. The specimens collected from the column were then analyzed on a spectrophotometer and the protein peaks representing thyroglobulin were pooled. The protein was then passed through a Sepharose 4B column for further purification. The specimens representing thyroglobulin were pooled and concentrated.

The purity of the thyroglobulin separation was assessed in several ways. Polyacrylamide gel electrophoresis was performed which showed a dense major band representing 19S thyroglobulin plus a faint band representing the 12S component. These bands fell in the identical positions of a standard preparation of thyroglobulin prepared earlier at the National Institutes of Arthritis and Metabolic Disease by Dr. Becker. A specimen of the purified thyroglobulin was analyzed in a Beckman sucrose density analytical ultracentrifuge. The results confirmed the presence of a protein with 90% of the total representing 19S and 10% representing 12S. This was exactly as expected for thyroglobulin. Further evidence of the purity of the protein will be discussed below.

The purified thyroglobulin was used to immunize four New Zealand white rabbits against human thyroglobulin. The rabbits have been injected with thyroglobulin plus Freund's adjuvent for 28 weeks as of June, 1976. Antibodies against human thyroglobulin were apparent in significant titer as early as 6 weeks after the immunization series began. At 12 weeks of immunization 20-30 milliliters of blood were removed from each rabbit for development of the radioimmunoassay. The remainder of this presentation deals with anti-thyroglobulin antibody obtained at this bleeding.

Immunological Purity

The immunological purity and the specificty of the protein

as well as the antibody produced in the rabbits, was assessed by a series of studies on Ouchterlony plates. Various concentrations of thyroglobulin and antibody were tested, as well as heterologous sera from horse and rabbits. In addition, the immunological cross reactivity was assessed by examining for possible reactivity with pure triiodothyronine (T-3) and pure thyroxine (T-4). The Ouchterlony plates showed distinct lines of precipitate with pure thyroglobulin against rabbit anti-thyroglobulin anti-serum, but there was no evidence of cross reactivity to horse serum, rabbit serum, pure T-3 or pure T-4.

Further assessment of the immunological purity was obtained by immunoelectrophoresis. Thyroglobulin was electrophoresed with normal human serum, horse serum, normal rabbit serum, rabbit antibodies raised against thyroglobulin, and concentrated serum from patients with a very high antithyroglobulin titer. The immunoelectrophoresis results show that there is a distinct specificity of the rabbit antithyroglobulin anti-serum for the human thyroglobulin prepared and used for immunization. In the same system, serum from patients with a high antithyroglobulin antibody titer reacted with this human thyroglobulin. The pattern of immunoelectrophoresis showed that there was no cross reactivity with horse serum or rabbit serum.

125-I labeled thyroglobulin was prepared in this Laboratory for use in the radioimmunoassay. The 125-I labeling was performed by a modification of the method of Hunter. Iodination was achieved using Chloramine-T as the oxidizing agent, and sodium metabisulfite was utilized to stop the reaction. 1mCi of 125-I was used for each preparation. 5mcg of thyroglobulin was iodinated. The iodinated thyroglobulin was found to have a specific activity of from 50-100 microcuries per microgram during various iodination procedures. This 125-I labeled thyroglobulin was used for the radioimmunoassay.

The radioimmunoassay was developed in stages. A double antibody dilution test series was performed to find the appropriate dilutions of carrier normal rabbit serum and sheep anti-rabbit gamma globulin which was used as the second antibody. After this was completed a 125-I thyroglobulin affinity test was performed which assessed the affinity of the rabbit antithyroglobulin antibody for the labeled thyroglobulin. This test showed that the labeled thyroglobulin was not damaged and had the same specificity as unlabeled thyroglobulin. An antibody titer study was performed next which showed that all four rabbit sera had excellent antibody titers but that serum from rabbit (#13) was the best. Anti-serum titration curves were

then produced which showed that the rabbit antithyroglobulin anti-serum was sensitive down to approximately 10 ng/ml but was not capable of separating 1 ng/ml from 10 ng/ml. Hopefully, anti serum obtained from subsequent bleeding will show greater sensitivity.

The specific methodology for the radioimmunoassay for thyroglobulin was next developed. A standard curve was performed using dilutions of pure thyroglobulin to form the standard samples. The dilution of first antibody was changed in successive runs to determine the optimal dilution. Currently, the first antibody dilution is being used in a final dilution of 1:2,000,000. The assay initially involved three incubation steps at 4°C and took one week to perform. Recent modifications utilize shorter incubation periods at 37°C which permit equivalent results in only 2 days of assay time.

A normal range for human serum thyroglobulin was established by analyzing the serum concentration in 30 normal volunteers. The 30 normals showed a thyroglobulin range from 7.5 to 29.3 ng/ml. The mean was 14.26 with a standard deviation of 5.07. The tentative "normal" will be taken as any value less than 25.0 ng/ml.

A considerable acceleration in the investigative aspects of the assay occurred in early May with the location and hiring of a well trained and accomplished biochemical technician. Great difficulty had been encountered in locating a person of sufficient experience and knowledge and since his arrival on the scene, progress in the technical aspects of the work has increased significantly. Although it is felt that the optimum procedures for the thyroglobulin radioimmunoassay are not yet achieved, a working assay, specific and sensitive is now in use. While work is going on in the development of an improved assay, an effective procedure is currently available and is being used clinically.

Preliminary Clinical Results

Because of the delays in the development of the radioimmunoassay, only limited clinical studies have been carried out. In addition, because of our inability to obtain equipment to measure thyroidal iodine content by fluorescence scanning part of the clinical study of hyperthyroidism was not possible and changes in the protocol were necessary. Thus, although some studies have been carried out on hyperthyroid patients

with rapid iodine turnover and therefore presumptive small thyroidal iodine pool, no data is available on these patients at present. A few studies have been carried out in hyperthyroid patients prepared for surgery with propranolol and without antithyroid drugs and these are presented below. The major clinical studies to date have been directed to the question of determining the normal values for the radioimmunoassay as well as collecting serum on patients receiving a variety of iodine contrast materials.

The first group of patients to be studied are those about to undergo elective thyroid surgery for various disorders who have been given small oral doses of iodine 24 hours prior to surgery. Serum thyroglobulin levels as well as the levels of all thyroid hormones have been assessed before the dose of iodine, 24 hours later, after surgery and several months later. Data is available on only a few patients.

One hyperthyroid patient prepared for surgery with propranolol alone had interesting results. The levels of thyroid hormones (T-4, T-3) and TSH showed no significant change following the administration of iodine. In striking contrast however, the serum thyroglobulin level was quite abnormal. The baseline (pre-iodine) level was very high, about 20 times normal. 24 hours after the administration of 250 mg of iodide in the form of SSKI, (but before surgery), the patient's serum thyroglobulin level had tripled to over 60 times normal. One day after subtotal thyroidectomy the serum thyroglobulin level had fallen back to its original baseline value, and several months later the serum thyroglobulin level was in the normal range. This appeared to demonstrate that in a susceptible gland a small dose of iodide triggered the release of a large amount of thyroglobulin.

Another patient series involves the use of iodine containing X-ray contrast materials. These iodine containing materials are used routinely for various X-ray procedures. We are attempting to accumulate a large series of patients who will have bloods drawn before (control) and 24 hours, four days, and three to four months after the administered organic iodide contrast material. The series of patients in this category is also limited thus far. In the patients studied in early June, the serum thyroglobulin level was normal at baseline and did not rise significantly after the administration of normal dose of contrast for coronary arteriogram. Additional patients are now under study.

Serial serum thyroglobulin levels are also being measured in a series of patients who have received radioactive iodine therapy for hyperthyroidism. Blood is withdrawn immediately prior to treatment, one day after treatment day and at 2 day intervals for the subsequent week. This series includes approximately 200 patients to date and is designed to reveal the pattern of thyroglobulin release from hyperthyroid glands after radioiodine and perhaps its correlation with type of gland morphology, antithyroid drug pretreatment, and other parameters of the treatment episode.