0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 11
Printed in U.S.A.

High Prevalence of Antineutrophil Cytoplasmic Antibody Positivity in Childhood Onset Graves' Disease Treated with Propylthiouracil

HIROKAZU SATO, MOTOSHI HATTORI, MIKIYA FUJIEDA, SHIGETAKA SUGIHARA, HIROAKI INOMATA, MARI HOSHI, AND SHIGEKI MIYAMOTO

Divisions of Endocrinology (H.S., S.M.) and Nephrology (M.Ha.), Chiba Children's Hospital, Chiba 266-0007; Department of Pediatrics, Kochi Medical School (M.F.), Kochi 783-8505; Department of Pediatrics, Daini Hospital, School of Medicine, Tokyo Women's Medical University (S.S.), Tokyo 116-0011; Department of Pediatrics, Ichihara Hospital, School of Medicine, Teikyo University (H.I.), Chiba 299-0111; and Department of Pediatrics, Matsudo Municipal Hospital (M.Ho.), Chiba 271-0064, Japan

ABSTRACT

Propylthiouracil (PTU)-induced antineutrophil cytoplasmic antibody (ANCA)-related vasculitis and nephritis were recently reported in about 30 patients with hyperthyroidism. The objective of this study was to clarify the prevalence of ANCA and the relationship between ANCA and thyroid antibodies in children with Graves' disease.

Titers of myeloperoxidase (MPO)-ANCA in sera of 51 patients with childhood onset Graves' disease (16 before treatment, 25 and 10 treated with PTU and methimazole, respectively) were measured by enzyme-linked immunosolvent assay. Antithyroglobulin antibodies (TGAbs) and antithyroperoxidase antibodies (TPOAbs) were also measured by RIA in 25 PTU-treated patients. No patients had clinical manifestations of vasculitis and nephritis. MPO-ANCA was positive in 6.7% of patients before treatment and in 64.0% of those treated with PTU and in none of those treated with methimazole. MPO-ANCA had a significantly positive correlation with TGAbs ($P < 0.05$) and no significant correlation with TPOAbs.

These findings show the high prevalence of the MPO-ANCA positivity in PTU-treated childhood onset Graves' disease, suggesting that PTU may not be preferred as the first line for the treatment of children with Graves' disease. The significant correlation between MPO-ANCA and TGAbs indicates that the severity of Graves' disease may be a factor responsible for the MPO-ANCA positivity. The cross-reactivity between MPO-ANCA and TPOAbs may not play a role in the high prevalence of MPO-ANCA in the patients exposed to PTU. (*J Clin Endocrinol Metab* **85:** 4270–4273, 2000)

ANTITHYROID DRUG therapy with thioamides, such as propylthiouracil (PTU) and methimazole (MMI), is commonly used in Graves' disease. Antithyroid drug therapy often accompanies several side effects, including a slight increase in liver enzymes, leukopenia, skin rash, and arthralgias (1, 2), although vasculitis is a rare complication. In 1992, Stankus and Johnson (3) first reported antineutrophil cytoplasmic antibody (ANCA)-positive PTU-induced hypersensitivity vasculitis presenting as respiratory failure, and Dolman *et al.* (4) reported detection of ANCA in serum from six patients who developed vasculitis during PTU treatment of hyperthyroidism in 1993. Since then, PTU-induced ANCA, especially myeloperoxidase (MPO)-ANCA-related vasculitis and nephritis have been reported in about 30 patients with hyperthyroidism (3–18), including three children (5, 17).

ANCA is present in sera of a large number of patients with systemic necrotizing vasculitis. These autoantibodies are directed at myeloid lysosomal enzymes and may be demonstrated in a cytoplasmic staining pattern or a perinuclear staining pattern by indirect immunofluorescence. The cytoplasmic ANCA directed at proteinase-3 (PR3-ANCA) is strongly associated with Wegener's granulomatosis (19). The perinuclear ANCA directed at myeloperoxidase (MPO-ANCA) is associated with microscopic polyarteritis nodosa, idiopathic necrotizing, and crescentic glomerulonephritis (20, 21).

In children with hyperthyroidism, antithyroid drug therapy is frequently chosen as the initial treatment to avoid surgery and possible long-term teratogenic or carcinogenic effects of radioiodine (22). However, most children require a relatively long period of medical treatment, and adverse reactions are more common in children (2). This raises the question: Is it appropriate to select PTU as the first choice for antithyroid drug therapy in children? Furthermore, no data are available in the literature on the prevalence of MPO-ANCA in children with hyperthyroidism. Therefore, we conducted a cross-sectional observational study for the prevalence of MPO-ANCA in childhood onset Graves' disease before and after antithyroid drug therapy and evaluated the correlation between the MPO-ANCA positivity and thyroid autoantibodies in PTU-treated patients.

Materials and Methods

Subjects

Fifty-one Japanese patients with Graves' disease (41 females and 10 males) were recruited for this study. Graves' disease was diagnosed between 3 and 15 yr of age (11.6 ± 2.4, mean ± SD) on the basis of clinical features, diffuse goiter, positive antithyroid antibodies, and hyperthyroidism, and they were followed at the Chiba Children's Hospital, Daini

Received January 28, 2000. Revision received August 2, 2000. Accepted August 8, 2000.

Address correspondence and requests for reprints to: Hirokazu Sato, M.D., Division of Endocrinology, Chiba Children's Hospital, 579-1 Hetacho, Midori-ku, Chiba 266-0007, Japan.

Hospital, School of Medicine, Tokyo Women's Medical University, Ichihara Hospital, School of Medicine, Teikyo University, and Matsudo Municipal Hospital. The selection of antithyroid drugs is a matter of the personal preference of pediatric endocrinologists in each hospital. The dosage of initial treatment with PTU and MMI was 10 mg/kg/day (maximum dose, 300 mg/day) and 1 mg/kg/day (maximum dose, 30 mg/day), respectively. The patients treated with both PTU and MMI in each for a different period, short as it was, were excluded from this study. None had clinical manifestations of vasculitis and nephritis. Informed consent was obtained from all patients and/or guardians involved in this study.

Subjects consisted of 16 patients before treatment (14 females and 2 males), 25 treated with PTU (19 females and 6 males), and 10 treated with MMI (8 females and 2 males). The mean age at diagnosis in the group before treatment was 11.9 ± 2.8 yr (4–15). In the PTU-treated group, 20 patients were receiving PTU and 5 patients were in remission after discontinuation of PTU at blood sampling. The remission period was 0.7, 3.0, 3.1, 4.7, and 18.3 yr. The mean period from diagnosis to blood sampling was 5.3 ± 5.1 yr (0.6–21.0), and the duration of PTU therapy was 4.0 ± 3.6 yr (0.6–17.5). A male patient with Down's syndrome and a female patient complicated with type 1 diabetes were included in this group. In the MMI-treated group, the mean period from diagnosis to blood sampling was 2.4 ± 2.8 yr (0.4–9.6), and the duration of MMI therapy was 2.1 ± 2.8 yr (0.4–9.6). Nine patients were receiving MMI, and one was in remission 1 yr after discontinuation of MMI at sampling. There were no significant differences in gender (χ^2 test), the period from diagnosis to blood sampling, and the duration of antithyroid drug therapy (unpaired t test) between the PTU-treated group and MMI-treated group.

All blood samples were immediately centrifuged, and supernatants were stored at −20C until assayed. Titers of MPO-ANCA were measured in all patients. PR3-ANCA, antithyroglobulin antibodies (TGAbs), and antithyroperoxidase antibodies (TPOAbs) were measured in the PTU-treated patients. After MPO-ANCA measurement, urine analysis was made in the MPO-ANCA-positive patients. Blood analysis including the white blood cell count and sedimentation rate was made in two patients with the titer of MPO-ANCA higher than 100 enzyme-linked immunosorbent assay (ELISA) units (EU). MPO-ANCA was also measured using stored serum 12 days after the start of PTU therapy in one patient with positive MPO-ANCA.

Measurement

Serum MPO-ANCA was measured with commercial ELISA kits (23). Ninety-six-well ELISA plates were coated with MPO purified from human neutrophil cytoplasmic α granule (Nissho, Kusatsu, Japan). Two hundred microliters of a 1:20 dilution of serum was added to each well and incubated in duplicate for 1 h at 25 C. After three washings, 200 μL/well of an appropriate dilution of alkaline phosphatase-conjugate antihuman IgG were added and left 1 h at room temperature. Plates were washed again three times, and the substrate was added to each well. The optical density was read at 405 nm with a microplate reader before and after a 45-min incubation in the dark at room temperature. Titers of MPO-ANCA were calculated from the differences of the optical density before and after incubation using the standard curve obtained from three attached standards (10, 100, and 1000 EU). The limit of detection in this assay was 10 EU. The intra-assay coefficient of variation was 2.54% at 28.3 EU, 7.38% at 177.1 EU, and 5.88% at 511 EU. The interassay coefficient of variation was 8.13% at 29.6 EU, 5.63% at 171.5 EU, and 7.96% at 584.4 EU. The sensitivity and specificity compared with perinuclear ANCA measured by indirect immunofluorescence assay were 97.4% and 92.0%, respectively (23).

Serum PR3-ANCA was measured with commercial ELISA kits using PR3 purified from human neutrophil cytoplasmic α granule (BioCarb Limited, Lund, Sweden). The limit of detection was 10 EU. The intra-assay and interassay coefficients of variation were 1.2–6.5% and 3.7–5.3%, respectively. There was a close correlation between serum PR3-ANCA measured by ELISA and cytoplasmic ANCA by indirect immunofluorescence assay (r = 0.979) (24).

Serum TGAb and TPOAb were measured with commercial RIA kits using purified TG and TPO, respectively (RSR Limited, Cardiff, UK). The detection limit of both was 0.3 U/mL. The intra-assay and interassay coefficients of variation of TGAb were 3.3–4.0% and 4.1–5.7%, respectively (25). The intra-assay and interassay coefficients of variation of TPOAb were 2.0–3.2% and 3.5–5.2%, respectively (25).

Statistical analysis

Values were expressed as the mean ± SD. The correlations between the titer of MPO-ANCA and the duration of PTU therapy, and the titer of TGAb and TPOAb were determined by Spearman rank correlation coefficient. Statistical calculations were made using StatView 4.5J software (Hulinks, Tokyo, Japan). Differences were considered significant at $P < 0.05$.

Results

MPO-ANCA in three groups (Fig. 1)

The MPO-ANCA positivity was detected in one (6.4%) of the patients before treatment, 16 (64.0%) of the PTU-treated patients, and none of the patients treated with MMI. In the PTU-treated MPO-ANCA-positive patients, the titer of positive MPO-ANCA ranged from 10–205 EU. Three of the 16 MPO-ANCA-positive patients were those examined after discontinuation of PTU therapy; the remission period in these three patients was 0.7 yr, 3.0 yr, and 18.3 yr, and the MPO-ANCA titer was 34 EU, 18 EU, and 28 EU. There was no significant correlation between the titer of MPO-ANCA and the duration of PTU therapy. MPO-ANCA was undetectable in a patient with Down's syndrome and a diabetic patient. The titer of positive MPO-ANCA in a girl before treatment was 10 EU, which was the minimum concentration of detection. Her condition of hyperthyroidism was moderate, and serum MPO-ANCA turned negative 2 months after the start of MMI therapy.

PR3-ANCA, TGAb, and TPOAb in PTU-treated group

The PR3-ANCA positivity was detected with the titer of 12 EU in one patient (4.0%). This patient had the highest titer of MPO-ANCA (205 EU). The TGAb positivity was detected in 21 (84.0%) patients, and the titer ranged from 0.3–303 U/mL. There is a significant positive correlation between titers of MPO-ANCA and TGAb (r = 0.71, $P < 0.05$, Fig. 2). The

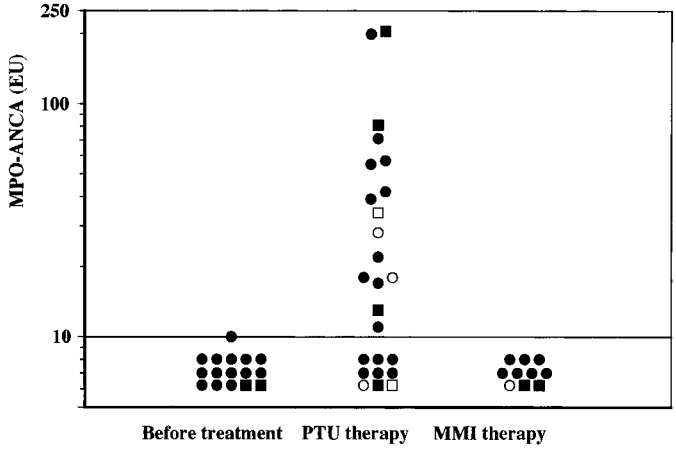

FIG. 1. Serum titers of MPO-ANCA in patients with Graves' disease before treatment, and those treated with PTU and MMI. The limit of detection is 10 EU. *Circles* and *squares* indicate female and male patients, respectively. *Open circles* and *squares* in treated groups indicate the patients who have already stopped taking the antithyroid drugs.

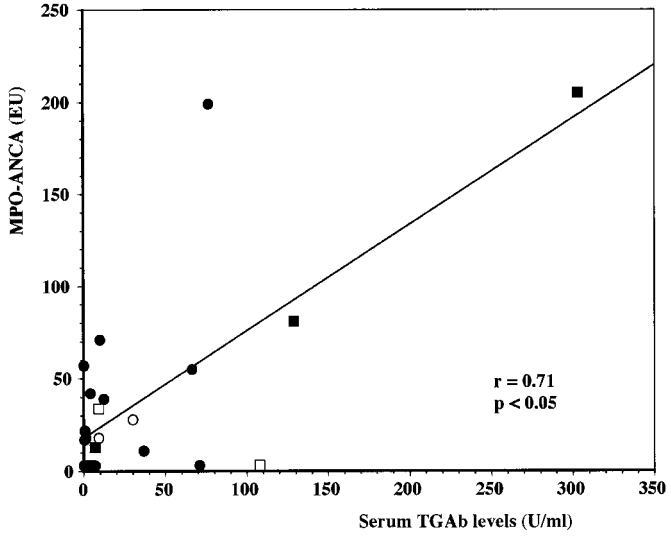

FIG. 2. The correlation between serum titer of MPO-ANCA and TGAb in patients with Graves' disease treated with PTU. The limits of detection are 10 EU of MPO-ANCA and 0.3 U/mL of TGAb. *Circles* and *squares* indicate female and male patients, respectively. *Open circles* and *squares* indicate the patients who have already stopped taking PTU.

TPOAb positivity was detected in 24 (96.0%) patients, and the titer ranged from 0.3–272 U/mL. No significant correlation was observed between the titers of MPO-ANCA and TPOAb (r = 0.14, $P = 0.43$). No significant correlation was observed between the titers of TGAb and TPOAb (r = 0.12, $P = 0.14$) either.

Laboratory findings in patients with positive MPO-ANCA

Microscopic hematuria was detected in two patients. Urinalysis had been carried out periodically since the start of PTU therapy in these patients. Although their microscopic hematuria was detected occasionally, proteinuria and macroscopic hematuria were never observed. White blood cell count and sedimentation rate in two patients with a high titer of MPO-ANCA (>100 EU) was within normal range. MPO-ANCA was negative in stored serum 12 days after the start of PTU therapy in one patient with positive MPO-ANCA (57 EU) after 1.48 yr of PTU treatment.

Discussion

The prevalence of the MPO-ANCA positivity in the PTU-treated patients was much higher than that in the MMI-treated patients. Because the selection between PTU and MMI for treatment is a matter of the personal preference of each doctor, factors that led to the development of positive MPO-ANCA for the PTU-treated group might be excluded. PTU was preferred to MMI because of the additional property of PTU in inhibiting the peripheral conversion of T_4 to T_3. However, agranulocytosis, the most feared side effect, occurs with equal frequency with PTU and MMI, and other major clinical side effects seem to be more common with PTU (26). MMI has become more preferred recently because of the patient compliance and quick response to initial therapy. Therefore, the mean period from diagnosis to blood sampling and the mean duration of treatment in PTU-treated patients tend to be longer than those in MMI-treated patients. MPO-ANCA was not detected in sera of patients before treatment, except one with the detection limit of ELISA. Furthermore, the development of MPO-ANCA-positive response during PTU therapy was confirmed in one patient. Our results strongly suggest that PTU by itself induces MPO-ANCA in patients with Graves' disease. Although the pathogenesis is not clearly understood, PTU has been shown to accumulate in neutrophils (27), bind to MPO, and change its structure (28). This alteration in configuration may allow initiation of the autoantibody formation.

Nearly half of the reported cases of PTU-induced ANCA-related vasculitis and nephritis were Japanese patients (18). It indicates that there may be a racial difference in the prevalence of MPO-ANCA positivity and ANCA-related vasculitis and nephritis in Graves' disease treated with PTU. Honda *et al.* (29) presented the incidence of ANCA in adult Japanese patients with Graves' disease in 1996. They measured ANCA by both indirect immunofluorescence and MPO-ANCA-specific ELISA and detected positive MPO-ANCA in sera of 10 of 52 patients treated with PTU (19.2%), 1 of 51 patients treated with MMI, and 0 of 13 patients treated with other therapy than antithyroid drugs (29). Although the number of subjects in our study was smaller than that in the study by Honda *et al.* (29), childhood onset Graves' disease is uncommon, accounting for less than 5% of all cases of Graves' disease (30). So, we can not make a simple comparison because of the variety of differences between these two works. The prevalence of MPO-ANCA positivity in our childhood onset patients treated with PTU was surprisingly higher than that of adult onset Japanese patients. This discrepancy, unclear as its cause is, may be attributed to the relatively high dose of PTU used in the childhood onset patients. The initial daily dose of PTU in children, 5–10 mg/kg, is usually higher than that in adults with 5 mg/kg (*e.g.* body weight, 60 kg). Sediva *et al.* (31) measured ANCA in a large amount of consecutive sera sent for the routine immunological investigation and speculated that the spectrum of ANCA-positive diseases differed somewhat between children and adults. Although MPO-ANCA was not detected in MMI-treated patients and the appearance of PR3-ANCA in PTU-treated patients was rare, cases of MPO-ANCA-positive glomerulonephritis associated with MMI (32, 33) and a case of PR3-ANCA-positive Wegener's granulomatosis receiving PTU for Graves' disease (34) also have been reported.

MPO-ANCA had a positive correlation with TGAb. Kasagi *et al.* (35) reported that TGAb measured by RIA could most precisely predict the histological findings of Hashimoto's thyroiditis in thyroid autoantibody. If the serum level of TGAb reflects the degree of thyroid destruction even in Graves' disease, it indicates that the severity of Graves' disease may be a factor responsible for the MPO-ANCA positivity in patients exposed to PTU. On the other hand, Becker *et al.* (36) reported the membranous glomerulonephritis associated with Graves' disease and postulated that the release of thyroglobulin or other antigens from the thyroid would lead to this disorder in autoimmune thyroid disease. Further examination is needed to clarify the significance of TGAb related to MPO-ANCA. On the other hand, TPO accounts for

almost all of the antigenic determinants recognized by microsomal antibodies in autoimmune thyroiditis (37). There is more than 40% structural homology between TPO and MPO (38). Although Haapala *et al.* (39) reported the cross-reactivity between antibodies to thyroid microsomal antigens and MPO, we find no significant correlation between MPO-ANCA and TPOAb in this study as reported by Honda *et al.* (29) in a study of adult patients with Graves' disease. This suggests that the cross-reactivity between MPO-ANCA and TPOAb is not playing a role in the prevalence of MPO-ANCA.

Despite the high prevalence of our MPO-ANCA-positive patients, none had clinical manifestation and laboratory findings of vasculitis and nephritis. Some other factors may be needed for vasculitis and nephritis to develop in MPO-ANCA-positive hyperthyroid patients on PTU therapy. Long-term follow-up will clarify the significance of this. MPO-ANCA-related nephritis, however, often causes an irreversible renal dysfunction after progressive crescentic glomerulonephritis (20, 21). Children with Graves' disease often require a prolonged course of antithyroid therapy (2), and side effects of MMI seem to be equal to or less common than those of PTU. Consequently, in our opinion, PTU may not be preferred as the first line for the treatment of patients with Graves' disease, especially in childhood onset patients, and should be used with a close observation on ANCA-positive patients.

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