Graves' Disease: Analytical Methods to Determine Antithyroid Therapeutic Agents in Biological Fluids

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Abstract

Graves' disease is an autoimmune disease arising from antibodies that attach to receptors on thyroid hormone-producing cells in the thyroid gland triggering off over production of thyroid hormone. The most important diagnostic tests for Graves' disease are measurements of serum levels of anti-TSH-receptor antibodies (TRAb) and thyroid ultrasonography. Clinical management of the disease involves the use antithyroid drugs, which mainly inhibit synthesis of thyroid hormone, or ablative treatments (iodine 131-radiotherapy or thyroidectomy) that remove or reduce thyroid tissue. Though these treatments do not target the disease process, however new agents blocking the thyrotropin and insulin-like growth factor receptors are under evaluation in preclinical or clinical studies for such purpose. Of all the analytical methods such as spectroscopy, electrochemical, electrophoresis and chromatography that have been used to determine antithyroid therapeutic agents (drugs) in biological fluids, high performance liquid chromatography seems to be the analytical method of choice.

Keywords: Graves' Disease; Antithyroid Drugs; Biological Fluids; Analytical Methods

Introduction

Graves' disease is an autoimmune disorder (body's immune system seeing healthy cells as foreign invaders and attacking them) characterized by hyperthyroidism, orbital disease (Graves' ophthalmopathy), skin changes (thyroid dermopathy) and rarely, fingertip and nail abnormalities (thyroid acropachy) [1-4]. It is the most common cause of hyperthyroidism. Graves' disease can affect people of any age however women in age range of 40 - 60 years are most at risk in developing the disease [5].

The pathogenesis of the disease involves the binding of the antibodies to TSH receptors (TRAb) on the surface of thyroid follicular cells, leading to continuous and uncontrolled stimulation of thyroid gland, resulting in excess synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) and thyroid hypertrophy [6].

An enzyme (peroxidase) produces these thyroid hormones by combining iodine with a protein called thyroglobulin. Research suggests that Graves' disease may be caused by a combination of genetic (approximately 80%) and environmental (approximately 20%) factors [7-9]. In genetically predisposed individuals, these factors contribute to the onset of the disease by breaking the mechanisms that result in immune tolerance. Such environmental risk factors include adequate iodine intake, cigarette smoking, infections, pregnancy, sex hormones and stress.

The major symptoms and physical signs of Graves disease include [10,11]:

(i)   Symptoms: Anxiety, dyspnea, fatigue, increased sweating, thirst and polyuria, menstrual disturbances in women (oligomenorrhea or amenorrhea), loss of libido, eye symptoms (swelling, pain, redness, double vision) insomnia, nervousness, palpitations, tremor, tiredness, muscle weakness, weight loss.

(ii)   Extrathyroidal physical signs: Acropachy, chemosis, localized dermopathy, proptosis (exophthalmos), double vision (extra-ocular-muscle dysfunction), optic neuropathy and ophthalmopathy.

(iii)   Physical signs of hyperthyroidism: Atrial fibrillation, cardiac failure, hair loss, Fine tremor, hyperkinesis, hyperreflexia, moist skin, palmar erythema, onycholysis, mental-status and mood changes (example mania or depression), muscle weakness, weight loss, systolic hypertension, tachycardia.

The diagnosis of Graves' disease is currently based on anti-TSH-receptor antibody assays and thyroid ultrasonography. Increased levels of free T4 and decreased levels of TSH is usually sufficient to confirm the diagnosis of Graves disease [12]. The serum level of TRAb is a useful laboratory test to determine whether Graves' disease is the cause of hyperthyroidism [13,14].

In the management of Graves' disease, the ideal treatment should be to restore normal thyroid function, prevent recurrence of hyperthyroidism, avoid development of hypothyroidism and prevent Graves ophthalmopathy progression [15]. These goals can be achieved by use of therapeutic agents (antithyroid drugs), radiotherapy (I131-radiotherapy) and thyroidectomy. Ablative therapies (I131-radiotherapy and thyroidectomy) induce lifelong hypothyroidism.

The thionamide derived antithyroid drugs namely methimazole, carbimazole (prodrug of methimazole) and propylthiouracil are the first-line drugs clinically used in the treatment of Graves' disease. These drugs act by decreasing excess thyroid hormone synthesis by inhibiting thyroid peroxidase (TPO), thereby reducing the production of T3 and T4 or indirectly through normalization of thyroid status in order to exhibit their immunosuppressive effects [16,17]. Propylthiouracil also blocks conversion of thyroxine to triiodothyronine.

These therapeutic agents can be administered by titration method (a variable starting daily dose is used, and the drug is then gradually decreased to the lowest dose that maintains serum levels of T4 in the euthyroid range) or block–replace method (a standard dose is administered together with a replacement dose of levothyroxine, to avoid hypothyroidism) [18]. Methimazole or carbimazole are the preferred thionamide antithyroid drugs while the use of propylthiouracil is reserved for patients intolerant to other thionamides and to women in the first trimester of pregnancy [19,20].

Other therapeutic agents used as adjunctive therapies are β-blockers or calcium-channel blockers. Beta-blockers that competitively block β-adrenergic receptors can be used during the initial phases of antithyroid drug treatment to reduce hyperthyroid symptoms [17].

Iodine 131 (I131)-radiotherapy is an effective therapy for Graves' disease and acts by causing gradual necrosis of thyroid cells and eventually results in hypothyroidism [21,22]. It is usually administered in fixed amounts or as calculated doses based on the estimated size of the thyroid and uptake of ^{131}I, 24h after administration.

Thyroidectomy is a considered an effective and definitive treatment for Graves' disease, however employed less often than I131-radiotherapy [23,24]. The procedure is clearly indicated in patients with relapse of hyperthyroidism after antithyroid drug treatment, those with large goiters and thyroid malignancy.

The objective of the present study is to provide analytical methods that determine first-line antithyroid drugs used in the management of Graves' disease in biological fluids. It is envisaged that the comprehensive information will assist clinicians and hospital-based pharmacists to know the readily available analytical methods to employ in monitoring biological fluid levels of these antithyroid drugs. Biological fluids are vital to life and assist in maintaining body homeostasis. Such biological fluids very often analyzed for drugs content include blood (whole blood, serum or plasma); urine; cerebrospinal fluid (CSF).

Effective treatment of disease is usually assessed by measuring the drug concentration(s) in the biological fluids [25]. In order to accurately quantify a drug in any biological fluid, measurement of drug concentration requires accurate, precise, sensitive, selective and specific analytical methods.

Literature search has provided a number of analytical methods that can be employed to determine antithyroid drugs used to treat Graves' disease and they include capillary zone electrophoresis [26], coulometry [27], high-performance liquid chromatography [28], spectroscopy [29], potentiometry [30] and thin layer chromatography [31]. Some of the analytical methods specifically applied to biological fluids include:

1. Methimazole (1-Methylimidazole-2-thiol):

    a. Human plasma:

        i. Meulemans., *et al*. [32] non-hyphenated chromatographic system (HPLC), amino (NH_2) column, complex formed between the drug and 2,6-dichloroquinone chlorimide was extracted in chloroform, detector was set at 405 nm and limit of detection was 5 ng/ml.

        ii. Floberg., *et al*. [33] hyphenated chromatographic system (GC/MS), one step derivatization by extractive alkylation using either benzyl chloride or pentafluorobenzyl bromide, deuterated-labelled methimazole was the internal standard and limit of detection was 5 ng/ml.

        iii. Skellern., *et al*. [34] non-hyphenated chromatographic system (HPLC), detection was at 254 nm.

        iv. Shekan., *et al*. [35] hyphenated chromatographic system (HPLC/IP-HFLPME), temperature was 45 deg C, calibration range was 0.5 - 1000 ng/ml and limit of detection was 0.1 ng/ml.

    b. Human urine:

        i. Zakrzewski [36] non-hyphenated chromatographic system (HPLC), detection at 350 nm, calibration range was 2 - 10 nmol/ml, limit of detection was 1 nmol/ml and limit of quantitation was 2 nmol/ml.

        ii. Kusinierek., *et al*. [37] non-hyphenated chromatographic system(HPLC), derivatization of drug with 2-chloro-1-methylquinolinium tetrafluoroborate to give methimazole 2-S-quinolinium derivative, detection was at 345 nm, calibration range was0.25 to 50 µg/ml, limit of detection was 0.15 µg/ml and limit of quantitation was 0.25 µg/ml.

2. Propylthiouracil: A number of analytical methods used to determine propylthiouracil in biological fluids include:

    a. Human plasma:

        i. Ringhand., *et al*. [38] non-hyphenated chromatographic system (HPLC), reversed-phase C_{18} chromatographic column, detection at 280 nm, calibration range was between 0.1 and 5.0 µg/ml and internal standard was methylthiouracil.

        ii. Connell., *et al*. [39] non-hyphenated chromatographic system (HPLC), the assay was linear to 3 µg/ml and limit of detection was 40 ng/ml.

      iii.   Giles., *et al.* [40] non-hyphenated chromatographic system (HPLC, protein precipitation was done with aceto-nitrile, columns were C_{18} and C_8 columns in series and calibration range was 0.25 - 10 µg/ml.

    b.   Human serum:

      i.   Ratliff., *et al.* [41] spectroscopic method, based on reaction of the drug with 2,6-dichloroquinone-chloroimide at pH 8.0 to give a colored chloroform-soluble compound, maximum wavelength was 435 nm and Beer's law was obeyed up to a concentration of at least 10 µg/ml.

Although the bioanalytical methods presented may not be exhaustive but are considered sufficient for clinicians and hospital-based pharmacists who very often monitor drug levels in biological fluids.

Conclusion

Graves' disease, an autoimmune disorder is the most common cause of hyperthyroidism. Management of the disease involves the use of antithyroid therapeutic agents, iodine 131 (I131)-radiotherapy and thyroidectomy. Determination of antithyroid therapeutic agents in biological fluids has been accomplished mostly by non-hyphenated liquid chromatography (HPLC). However, hyphenation of the chromatographic system with spectroscopic system has also been used to enhance accuracy, precision sensitivity and selectivity.

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