Advances in the Treatment of Graves' Disease – A Focus on Rituximab

a report by

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Graves' disease is the most common cause of hyperthyroidism. The incidence is 5/1,000 per year, with a female-to-male ratio of 7:1.[1,2] Graves' disease is caused by autoantibodies that bind to and stimulate the thyrotropin (TSH) receptor on thyroid follicular cells, thereby stimulating thyroid hormone synthesis and secretion as well as thyroid growth. The pathogenesis of Graves' disease is multifactoral, including genetic, environmental and endogenous factors.[3-6]

Current therapies of Graves' disease are aimed at reducing thyroid hormone synthesis and consist of thionamides, radioiodine and surgery.[6] Thionamides block the thyroid hormone synthesis by inhibition of thyroid peroxidase, and are usually prescribed for at least one year. The major disadvantage of thionamides is the low remission rate of only 30-40% of patients after 10 years.[7,8] In addition, rare but serious side effects of thionamides, such as agranulocytosis and hepatotoxicity,[8] limit prolonged use of these drugs. Radioiodine therapy is indicated for relapsing Graves' disease in Europe and Japan, whereas this is the treatment of choice in first episodes of Graves' disease in the US. Hypothyroidism is the major complication of radioiodine therapy with an estimated incidence of 30% in the first two years after therapy and thereafter a yearly incidence of 5%.[9,10] Surgery is not used often as a treatment for Graves' disease and is mainly restricted to patients with obstructive goitre, opthalmopathy and uncertain histology of nodes and patients refusing radioiodine therapy. Obvious complications of surgery are lesions of the n recurrens and hypothyroidism. Obviously, current therapies are not aimed at the underlying pathogenetic mechanisms in Graves' disease. The imperfections of current therapies for Graves' disease substantiate the need for new treatment options aimed at the pathology of Graves' disease.

Rituximab

Auto-antibodies directed against the TSH receptor are crucial in the pathogenesis of Graves' disease. These antibodies are synthesised by infiltrating B cells into the thyroid. Plasma cells from the bone marrow and cervical lymph nodes are also involved in the synthesis of autoantibodies.[11–13] Therefore, a promising strategy for treating Graves' disease would be the elimination of activated B-lymphocytes. Pre-B-lymphocytes as well as activated mature B-lymphocytes express CD20 on the surface,[14] but this expression is lost following differentiation into plasma cells. CD20 is also expressed on some pro-B-cells, the precursors of pre-B-cells, although in low numbers.[15,12] This makes the CD20 antigen an attractive target for treating Graves' disease. Moreover, anti-CD20 therapy could also interfere with the B-cell antigen-presenting role to T cells and thereby interfere with T-cell activities.[16]

Rituximab is a chimeric monoclonal antibody specific for human CD20.[14,17,18] Rituximab was originally used for the treatment of non-Hodgkins lymphoma.[19,20] It has subsequently been successfully used in the treatment of various autoimmune diseases,[21] including idiopathic thrombocytic purpura,[22] systemic lupus erythomatosus,[23,24] haemolytic anaemia[25] and rheumatoid arthritis.[16,26,27] Rituximab causes an immediate depletion of circulating B cells. This depletion lasts for four to six months, but may last for more than 24 months.[12,28,29] Pre-treatment levels of B cells will be reached after nine to 12 months. Rituximab kills B cells by induction of apoptosis by altering calcium influx,[30] antibody-dependent cellular toxicity and complement-dependent cellular cytotoxicity.[12]

Graves' Hyperthyroidism

Several studies have investigated the effect of treatment of Graves' disease with rituximab. Infusions with rituximab resulted in a decrease in CD-20+ B cells in all patients. Salvi et al. treated nine patients with Graves' disease with two infusions of rituximab 1g at a two-week interval.[31] At baseline, four patients were hyperthyroid and five were euthyroid (two were on methimazole treatment, two were in remission and one was receiving thyroxin treatment after previous thyroidectomy). Of the four patients who were hyperthyroid at baseline, three had persistent hyperthyroidism after infusions with rituximab. These patients received methimazole. One of the hyperthyroid patients had subclinical hyperthyroidism and became euthyroid eight months after treatment with rituximab. The euthyroid patients without medical treatment remained in remission. In one patient who had received methimazole, hyperthyroidism developed rapidly after discontinuation of methimazole, despite B-cell depletion. Therefore, Salvi et al. concluded that thyroid function is not affected by treatment with rituximab.

El Fassi et al. performed a prospective controlled trial in 20 patients.[32] Most patients had a first episode of Graves' disease. Ten patients were given four infusions with 375mg/m^2 of rituximab with a weekly interval, whereas the other 10 received no rituximab. All patients were treated with methimazole
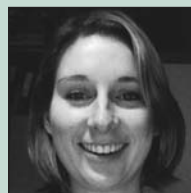
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until the last infusion with rituximab. Methimazole treatment was thereafter discontinued in all patients. In the 10 patients who were treated with the combination of rituximab and methimazole, six relapsed within one month after discontinuation. In the 10 patients who were treated with methimazole alone, eight relapsed within one month, one within three months and one after 13 months. However, methimazole treatment in this study lasted for around four months, which is unusually short. Therefore, it is not clear whether rituximab would be more efficacious compared with a full course of methimazole therapy.

Heemstra et al. studied 13 patients with relapsing Graves' disease.[33] All patients were treated with two infusions with of rituximab 1g with a two-week interval. Four patients did not respond to treatment with rituximab, whereas free T4 (FT4) levels decreased and TSH levels increased in nine. Median follow-up was 18 months (range 14–20 months). Non-responders had significantly higher FT4 levels than responders. El Fassi et al. found no differences in FT4 levels between responders and non-responders.[32] However, in that study patients were treated with methimazole before treatment with rituximab, whereas in the study of Heemstra et al. methimazole treatment had to be discontinued at least four weeks prior to treatment with rituximab to verify the existence of hyperthyroidism. El Fassi et al. found also a significant difference in thyrotropin-binding inhibiting immunoglobulin (TBII) levels between responders and non-responders, with patients with lower TBII levels responding more favourably to rituximab.[32] In addition, in the study by Heemstra et al. it seems that two non-responding patients had higher TBII levels than responders. It would be useful to study this further to define a subgroup of patients likely to be responders.

Auto-antibodies

Controversy exists about the effect of rituximab on TBII levels. No studies found a relationship between proportions of CD-20+ lymphocytes after rituximab treatment and TBII levels.[31–33] Salvi et al. observed no significant differences in TBII levels after infusion with rituximab,[31] whereas Heemstra et al. found a significant decrease in TBII levels after treatment with rituximab.[33] This decrease was observed only in the responders. El Fassi et al. also found a decrease in TBII levels after rituximab treatment.[32] However, this decrease was no different to that seen in patients treated with methimazole alone. Hoever, they did show a decrease in the TSH receptor stimulatory capacity of TBII with no change in absolute TBII levels in patients treated with rituximab and methimazole compared with patients treated with methimazole alone, suggesting an alteration of the balance from stimulating into non-stimulating TBII due to decreased production of stimulating TBII.[34] The association between circulating levels of CD20+ lymphocytes, serological markers of autoimmune disease and effectiveness of rituximab in autoimmune disease is complex; however, several studies in patients with rheumatoid arthritis and lupus imply an inverse relationship between proportions of B cells, autoantibody titers and therapeutic effect.[16,23,35,36]

Explanations for a Lack of Effect of Rituximab

Synthesis of new antibodies may occur after rituximab treatment given that the half-life of human immunoglobulin G (IgG) is approximately three weeks. In addition, it has been reported that the levels of immunoglobulin A and G remain unchanged after treatment with rituximab.[16,34] Another explanation may be that plasma cells that do not express CD20 are an important source of auto-antibodies. Moreover, a portion of plasma cells have a very slow turnover.[37] Furthermore, lymphoid cells in germinal centers in the thyroid may exhibit less pronounced B-cell depletion.[38] However,

El Fassi et al. showed complete intrathyroidal B-lymphocyte depletion after treatment with rituximab.[39]

Graves' Ophthalmopathy

Graves' disease is also associated with extrathyroidal complications, of which Graves' ophthalmopathy (GO) is the most prevalent. GO involves inflammation and swelling of retrobulbar fibrous and adipose tissue and extraocular muscles, causing mild to severe symptoms that include proptosis and compression of the optic nerve, affecting quality of life.[40] The pathogenesis of GO is not completely understood, but involves an immunological cross-reaction between antigens of the thyroid and orbital tissues, and includes pathological hyperactivation of orbital fibroblasts, deposition of collagen and glycosaminoglycans in the extracellular matrix and, eventually, fibrosis.[41] T cells also play a role in the pathogenesis of GO. A product of activated T cells, leukoregulin, stimulates the production of hyaluronan, prostaglandin-endoperoxidase H and proteins produced by orbital and pre-tibial fibroblasts.[42,43,44]

Ho et al. investigated the expression of multiple therapeutic targets in tissue specimens of 16 patients with orbital inflammation and found that CD20 was strongly expressed in these tissues, suggesting that it is a good target for the treatment of orbital inflammatory syndromes.[45] Indeed, several studies have investigated the effects of rituximab on GO. Salvi et al. reported a case in which one patient with GO that was unresponsive to steroid immunosuppression was treated with rituximab.[46] The clinical activity score of the GO improved, while hyperthyroidism remained. El Fassi et al. reported two patients with severe GO unresponsive to glucocorticosteroid therapy who were treated with rituximab.[47] The first patient remained euthyroid, whereas the second had persistent hyperthyroidism. In both patients GO improved. Salvi et al. also performed an open pilot study in which nine patients with GO were treated with two infusions of rituximab at a two-week interval and were compared with 20 patients treated with 16 weekly infusions of glucocorticosteroids.[31] The clinical activity score (CAS) and proptosis after treatment with rituximab improved significantly, whereas thyroid function was not affected. No patients developed relapse of active GO. In the glucocorticosteroids group, CAS and proptosis also improved significantlty. However, patients experienced more side effects after treatment with glucocorticosteroids and four did not respond to treatment with glucocorticosteroids. Two patients showed relapse six to eight weeks after the discontinuation of glucocorticosteroids infusions. In addition, improvement of the CAS was more pronounces in patients treated with rituximab than in those treated with glucocorticosteroids. Patients treated with rixumab showed improvement of the CAS within month of treatment, whereas patients treated with glucocorticosteroids showed improvement six to eight weeks after treatment.

Side Effects and Complications

Rituximab is safe in the majority of patients, although serious adverse events occur in a small minority.[14,48] The overall incidence and severity of adverse events after rituximab infusion is lower in patients with rheumatoid arthritis than oncologic patients. This may be the result of the absence of the cytokine release syndrome associated with tumour cell lysis.[14,48] Infusion-related-reactions consist of flu-like symptoms such as fever, chills and rigors, and occur within a few hours of starting rituximab infusion. Less common symptoms are hypotension, bronchspasm, pruritis and rash. Nevertheless, the majority (97 %) of these reactions were grade 1–2 according to the National Cancer Institute (NCI) Toxicity Scale.[14] Pre-medication consisting of

Table 1: Overview of the Literature on the Use of Rituximab in Graves' Disease

Author	Patients (n)	Treatment	Design	Outcome Thyroid Function	Outcome Graves' Ophthalmopathy
Salvi et al.[46]	1	2 infusions of rituximab 1g with 2-week interval	Case report		CAS improved.
El Fassi et al.[47]	2	375mg/m^2 riruximab weekly for 4 weeks and 2 months methimazole, discontinued at last RTX infusion	Case report	1 patient remained euthyroid and 1 patient remained hyperthyroid after methimazole discontinuation	CAS improved Proptosis improved in one patient in both eyes and in 1 patient only in the right eye
Salvi et al.[31]	9	2 infusions of rituximab 1g with 2-week interval	Prospective study	No effect on thyroid function	CAS, NOSPECS class 2, NOSPECS class 4 and proptosis improved CAS more pronounced and earlier improvement in rituximab group compared with glucocorticosteroids group
	20	16 weekly infusions with glucocorticosteroids			
El Fassi et al.[32]	10	375mg/m^2 rituximab for 4 weeks and methimazole ± 3–4 months, discontinued at last RTX infusion	Prospective controlled study	4 patients remained in remission in rituximab group All patients relapsed in methimazole-alone group	Only 2 patients with GO (47): CAS improved Proptosis improved in 1 patient in both eyes and in 1 patient only in the right eye
	10	Methimazole for ± 3–4 months			
Heemstra et al.[33]	13	2 infusions of rituximab 1g with 2-week interval	Prospective study	9 patients became euthyroid 4 patients did not respond	No patients with GO

CAS = clinical activity score; GO = Graves' ophthalmopathy.

paracetamol, antihistamines and corticosteroid may be useful to prevent these infusion-related reactions.[48,14] The frequency or severity risk of infections after rituximab infusions was not increased in a phase II study in patients with non-Hodgkins lymphoma.[49] However, pooled data show an increased risk of infectious events after rituximab infusion, but the risk of severe infections was low.[14] The low incidence of infections is probably due to the unaffected immunoglobulin levels.[14] The US Food and Drug Administration (FDA) also reported an increased risk of progressive multifocal leucoencephalopathy in patients treated with rituximab infusions (www.fda.gov/CDER/Drug/InfoSheets/HCP/rituximab.pdf).

In patients with Graves' disease treated with rituximab, mild infusion related reactions occurred in five out of 10 patients[32] and three out of nine patients[31] In the study by Heemstra et al., two patients developed temporarily joint complaints,[33] whereas in the study by El Fassi et al., two patients developed a serum-sickness-like reaction with joint pain and fever.[32] In the latter, treatment with rituximab was discontinued in two patients. In addition, El Fassi et al. reported the occurrence of

ulcerative colitis shortly after treatment with rituximab in a patient with irritable bowel syndrome.[50]

Concluding Remarks

We conclude that rituximab is probably effective in improving GO in patients who are unresponsive to treatment with glucocorticosteroids. Despite the high cost and side effects, rituximab may play a role in the treatment of severe active GO. Rituximab may also have a beneficial effect in Graves' hyperthyroidism. However, the results in these patients are less clear and efficacy should be confirmed with a randomised controlled trial. The design of such a study is nevertheless complex, because many confounding factors influence the outcome of these patients.[51] Complex issues in such trial are whether or not to pre-treat patients with thyreostatica drugs and the problem of blinding in studies involving radioiodine therapy. Nevertheless, the introduction of new classes of immunomodulatory drugs such as rituximab offer exciting new perspectives in the development of new approaches for the treatment of Grave's disease. ∎

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