Adjuvant Treatment of Graves' Disease with Diclofenac: Safety, Effects on Ophthalmopathy and Antibody Concentrations

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Key Words

Diclofenac · Anti-TPO · TSH receptor antibodies · Smoking

Abstract

Background: Orbital morphological changes are often present in patients with Graves' disease (GD) already at diagnosis, and cyclooxygenase type 2 (COX-2) is overexpressed in active Graves' ophthalmopathy (GO). **Objective:** To investigate if adjuvant treatment of GD with the COX inhibitor and peroxisome proliferator-activated receptor- γ (PPAR- γ) antagonist diclofenac decreases the development of ophthalmopathy and if laboratory parameters are affected. **Methods:** This is a multicenter trial where 61 subjects were randomized to methimazole (block and replace with L-thyroxine) either with or without diclofenac 50 mg 1 $\times$ 2 for 12 months. The primary end point development of GO after 24 months was evaluated. Smoking habits were registered and the thyroid parameters TSH, free T₄, free T₃, TSH receptor antibodies (TRAb) and anti-TPO were followed. Safety parameters (kidney, liver and blood) and adverse events were regularly registered. **Results:** GO developed in 11% (n = 3) of the patients treated with diclofenac and in 21% (n = 6) of the controls (p = 0.273). The adverse event profile was accept-

able without any severe events related to diclofenac. Both TRAb and anti-TPO concentrations decreased during treatment with methimazole, but the anti-TPO concentrations were lower in patients treated with diclofenac after 15 months (p = 0.031). The TRAb concentrations were not significantly changed between groups. Smokers had higher concentrations of TRAb than nonsmokers both at diagnosis of GD (p = 0.048) and after 15 months (p = 0.042). **Conclusions:** Treatment with diclofenac had no significant influence on development of GO. Diclofenac reduces anti-TPO concentrations and seems to be safe to use in GD patients.

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Introduction

In Graves' disease (GD), signs and symptoms from the eyes, i.e. Graves' ophthalmopathy (GO), appear in approximately one third of the patients at some point during the disease process [1]. Severe GO develops in 5% of the patients with GD. When performing MRI/CT/ultrasound of the orbital room, 98% of the patients with GD exhibit changes in the orbit without clinical symptoms of GO [1]. Thus, almost all patients with GD are at risk of

Table 1. Clinical characteristics at diagnosis of GD

Treatment	Diclofenac	Control
Number of subjects	30	31
Age, years	36±9.5	42±7.7
Female	80 (30)	87 (31)
Current smokers	57 (12)	43 (9)
Nonsmokers	47 (14)	53 (16)
Previous smokers	40 (4)	60 (6)
Born in Sweden	68 (17)	65.4 (17)
Born in Europe outside Sweden	20 (5)	23 (6)
Born outside Europe	12 (3)	11.5 (3)
TRAb, IU/l	8.1±5.8	8.5±8.6
TSH, mIU/l	0.03±0.05	0.05±0.08
Free T ₄ , pmol/l	37.1±17.4	37.1±17.0
Free T ₃ , pmol/l	15.7±9.6	15.0±9.6
Body weight, kg	67.6±14.2	64.8±11.6
Height, cm	172±9.5	167±7.7

Values are n (%) or means ± SD. Differences between groups were not significant. Data on ethnic background was only analyzed in patients included in Malmö (n = 51).

developing clinical GO. Environmental factors are important, and two strong risk factors for development of GO are smoking and treatment with radioiodine [2–6]. An interesting observation was made in a patient with inactive and stable GO, who developed active clinical ophthalmopathy when treated with pioglitazone due to type 2 diabetes [7]. In addition, it has also been described that treatment with pioglitazone increases eye protrusion in a subgroup of patients with type 2 diabetes [8]. One pathogenic mechanism in GO is increased orbital adipogenesis, and glitazones are known to increase the volume of subcutaneous adipose tissue [9]. Orbital fibroblasts from patients with ophthalmopathy have been shown to differentiate to adipocytes in response to rosiglitazone [10]. Glitazones are peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists and it is therefore of interest to study if PPAR- γ antagonists have preventive effects on the development of GO. Diclofenac has been shown to interact with PPAR- γ in physiological concentrations and to antagonize PPAR- γ -mediated effects like adipogenesis of the preadipocyte cell line 3T3-L1 [11, 12]. The natural ligand of PPAR- γ is prostaglandin J [13]. Therefore, non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac may affect both synthesis of prostaglandins and concomitantly antagonize the effects of the natural ligand to PPAR- γ , prostaglandin J₂. We have demonstrated upregulation of immediate early genes, including cyclooxygen-

ase type 2 (COX-2), which have important functions in the pathogenesis of adipogenesis in patients with severe ophthalmopathy [14]. There have only been two studies published on treatment of ophthalmopathy with NSAIDs (indomethacin or diclofenac) [15, 16]. Although only 7 patients were studied by Amemia [15], there were effects of indomethacin on soft tissue symptoms, eye protrusion and eye muscle symptoms. In a recent study by Bloise et al. [16], similar effects of diclofenac were shown on muscle symptoms and ocular pain. Due to the knowledge about retrobulbar morphological changes in patients with Graves' hyperthyroidism without clinical ophthalmopathy, it may be of importance to intervene as early as possible. The aim of this trial was to investigate if adjuvant treatment of GD, with diclofenac, decreases development of clinical ophthalmopathy, and determine if it is safe and if activity parameters are affected.

Material and Methods

Study Design

This study was designed as a prospective randomized multicenter trial in Malmö and Stockholm to compare 12 months of treatment with or without diclofenac (Diclofenac-Ratiopharm®) 50 mg twice a day in addition to medical treatment for GD. The primary end point was development of clinical GO 24 months after diagnosis of GD. The study was approved by the Swedish Medical Product Agency (EudraCT No. 2005-000832-26), and was registered in the Clinical Trials.gov protocol registration system (NCT01458600). It was approved by the Ethical Review Board of Lund University (Malmö/Lund, Sweden) and performed according to the Helsinki Declaration.

The standard 18-month treatment with antithyroid drugs was given with methimazole (Thacapzol®, Recip) 15 mg twice a day to block the synthesis of thyroid hormones and replace thyroid hormones by L-thyroxine (Euthyrox®, Merck) 100 µg once a day starting 3 weeks after methimazole. The L-thyroxine dose was adjusted to reach euthyroidism defined by normal and stable levels of free T₃, free T₄ and TSH during concomitant treatment with methimazole for 18 months. Effects of diclofenac on patient safety and laboratory parameters were registered.

Patients

Altogether 61 GD patients were included: 30 were randomized to adjuvant treatment with diclofenac and 31 were controls. Basal clinical characteristic are shown in table 1. All patients signed informed consent, which was obtained before inclusion.

The diagnosis of hyperthyroidism was based on clinical symptoms and signs, plasma concentrations of TSH <0.2 mIU/l, and increased plasma levels of free T₄ and/or free T₃. The patients were classified as having GD on the basis of clinical signs, the presence of TSH receptor antibodies (TRAb) and/or a diffuse uptake on thyroid technetium scintigraphy. Out of the 61 included patients, 5 were negative for TRAb, and out of these there were 2 patients negative for anti-TPO.

Inclusion criteria were: age 18–75 years and newly diagnosed GD without clinical GO. Exclusion criteria were pregnancy, previous treatment of thyroid disease, hypersensitivity to NSAIDs or acetylsalicylic acid, congestive heart failure, decreased renal function (creatinine >100 mmol/l), ASAT or ALAT >2.5 times the upper limit, coagulopathy, thrombocytopenia, previous or active gastric ulcer, inflammatory bowel disease, and clinical GO. Patients who met the inclusion criteria and lacked the exclusion criteria were randomized to treatment with or without diclofenac. Randomization was performed in blocks of 6 within each center.

The presence of GO was made by an ophthalmologist before inclusion and based on the presence or absence of clinical signs defined as one or more of the following: (a) decrease in visual acuity by at least two lines from baseline in any eye due to optic nerve involvement, (b) increase in exophthalmometry readings by more than 2 mm from base line in any eye, (c) eyelid edema in any eye, (d) conjunctival injection in any eye, (e) chemosis in any eye, (f) eye muscle involvement with restriction of motion in any direction and (g) corneal ulceration, as previously described in detail by Träisk et al. [6]. Eyelid retraction alone was not classified as GO and neither were symptoms alone of GO used in the classification of the presence or absence of ophthalmopathy. Patients with GO signs were not eligible for inclusion. Photos of all patients were taken at inclusion for comparison at follow-up and the ophthalmologist was blinded to choice of adjuvant treatment or not with diclofenac. All included patients were newly diagnosed with GD without previous treatment with antithyroid drugs. Enrolment started in September 2006 and ended in May 2012. Patients admitted to the two centers were continuously included, but the inclusion frequency was low in certain periods. In general, during screening half of the patients could be included and only 6 patients were lost during the 2-year follow-up period; the study was terminated in May 2014.

Assays

Plasma TSH (reference interval: 0.4–3.7 mIU/l, sensitivity: 0.001 mIU/l, CV: 10%), free T₄ (reference interval: 8–14 pmol/l, sensitivity: 2 pmol/l, CV: 10%) and free T₃ (reference interval: 3.5–5.4 pmol/l, sensitivity: 2 pmol/l, CV: 10%) were measured with the ELISA technique according to the manufacturer's instructions (Beckman-Coulter). TRAb were measured using a human radio-receptor assay kit purchased from Brahms following the manufacturer's instructions (reference interval: <1 IU/l, sensitivity: 0.3 IU/l, CV: 9.3–15.4%). The method has been used in clinical routine since April 2004. Anti-TPO titer was measured with the sandwich ELISA technique (Diagnostic Products Corporation) according to the manufacturer's instructions (normal reference interval: <35 kIU/l, sensitivity: 5 kIU/l, CV: 6%).

Safety and thyroid laboratory samples were analyzed in clinical routine laboratories at the Department of Clinical Chemistry in Malmö and Stockholm.

Statistical Analysis

In the power calculation, the number of patients needed in each group when performing a two-sided significance test at the 5% level is 72 to achieve 80% power to get significant results if the real difference is 0.2 (0.3 vs. 0.1). Significance testing of differences between the two study groups was performed using Student's *t* test for mean values and χ^2 test (Pearson) for proportion. *p* < 0.05 was deemed statistically significant. All statistical analyses were carried out using the SPSS 20.0 statistical software (SPSS, Chicago, Ill., USA).

Table 2. Development of ophthalmopathy after 24 months in patients treated with or without diclofenac

	Treatment with diclofenac, n	Controls, n	Total, n
GD patients with development of GO ¹	3	6	9
GD patients without development of GO	23	22	45

¹ *p* = 0.273.

Table 3. Number of patients with adverse events during treatment with methimazole and diclofenac (+) or without (–) diclofenac

Treatment diclofenac	+	–
Skin reactions	3	1
Neutropenia	0	3
Loss of taste	1	3
Joint pain/swollenness	0	1
Liver laboratory parameters	1	0
Gastrointestinal benign symptoms	5	0

Results

In this prospective multicenter study, patients with newly diagnosed GD without clinical ophthalmopathy were treated with methimazole and randomized to adjuvant treatment with diclofenac or not. The primary end point was to investigate if 12 months of treatment with diclofenac prevents or decreases development of clinical GO after 24 months. We found 9 cases with GO, and out of these 3 were treated with diclofenac and 6 were not (*p* = 0.273; table 2), but this difference did not meet statistical significance.

The two groups were comparable according to age, gender, smoking status, ethnic background and activity in hyperthyroidism (table 1). These two groups were carefully monitored and safety laboratory parameters were controlled at each visit during the 12-month treatment period with diclofenac (data not shown). In the aim to detect if and how the kidney function is affected, plasma creatinine was followed and shown to increase similarly in both groups. Liver function tests were also similar between groups at all visits except the 3-month visit when ALAT was occasionally increased in the group treated with diclofenac. Systolic and diastolic blood pressure did

Fig. 1. Effects of diclofenac on anti-TPO concentrations. **a** Mean anti-TPO at inclusion, after 9 months and 15 months. **b** Proportion of baseline anti-TPO concentrations after 9 and 15 months.

not change during the treatment period. The levels of blood status parameters (hemoglobin, thrombocytes and granulocytes) were stable during the 12-month period except in the 3 patients in the control group who developed neutropenia judged to be related to treatment with methimazole (table 3). Other methimazole-related adverse events reported were skin reactions, loss of taste and joint pain/swollenness. None of these events differed significantly between the groups. Gastrointestinal symptoms were strictly related to treatment with diclofenac, and in the majority of cases occurred during the first 2 weeks of treatment.

The number of patients who needed an adjustment of the L-thyroxine dose differed between the two groups. In addition, the patients treated with diclofenac required more dose changes and needed a higher dose of L-thyroxine (data not shown). This observation may reflect that the activity in the thyrotoxicosis is lowered by diclofenac. Therefore, we separately analyzed concentrations of anti-TPO and TRAb in plasma saved from each patient with increased concentrations of both antibodies. Anti-TPO showed lower concentrations after 9 and 15 months and the proportion of baseline concentrations was significantly lower ($p = 0.031$) in the group treated with diclofenac (fig. 1). This effect was not demonstrated for TRAb (data not shown). However, when comparing smokers

and nonsmokers at diagnosis of thyrotoxicosis, the proportion of smokers was lower in patients with low TRAb (≤ 2 IE/l) and higher in patients with TRAb > 2 ($p = 0.048$; fig. 2). It was also observed that TRAb persisted at higher concentrations after 9 and 15 months and that the proportion of baseline concentrations was significantly higher in smokers ($p = 0.042$).

Discussion

The TSH receptor and TPO are two major antigens associated with the autoimmune inflammatory reaction in both GD and GO. We have previously shown an overexpression of COX-2 in orbital tissue from GO patients [12]. Approximately one third of the patients with GD will develop clinical GO, but markers for patients at risk are lacking. The NSAID diclofenac has been given for 12 months as an adjuvant treatment in patients with newly developed Graves' hyperthyroidism. The proportion of patients without clinical GO was lower in the group treated with diclofenac, but did not meet statistical significance, probably due to the low number of patients who developed GO in both study groups. The low number of patients developing GO in those treated with antithyroid drugs is in line with a recent study on the natural course

Fig. 2. Effects of smoking on TRAb concentrations. **a** Proportion of smokers (black bars) and nonsmokers (gray bars) with TRAb over and under 2 IU/l at inclusion. **b** Mean TRAb at inclusion after 9 and 15 months. **c** Proportion of baseline TRAb concentrations after 9 and 15 months.

of GO [17]. We have found the adverse event profile acceptable without any severe events coupled to diclofenac. Both TRAb and anti-TPO decreased during treatment with antithyroid drugs, but the concentration of anti-TPO was significantly lower in the diclofenac group after 15 months, which was in contrast to the TRAb concentration that was not changed in response to diclofenac. On the other hand, smokers had higher concentrations of

TRAb than nonsmokers both at diagnosis of GD and after 15 months of treatment with antithyroid drugs. The thyroid activity in GD seems to be lower in patients treated with diclofenac since the replacement dose of L-thyroxine was higher and the number of dose changes was significantly increased in the diclofenac-treated group. An alternative explanation may be that diclofenac influences the absorption of L-thyroxine. It may seem paradoxical

that the decrease in TRAb titers does not follow the activity, but in a study where GD patients with ophthalmopathy were depleted of their peripheral B cells by use of rituximab (anti-CD20), the concentration of TRAb was found unchanged but the GO activity was reduced [18]. Immunoglobulin production in normal human B lymphocytes has been studied in vitro after activation by the CD40 ligand and it was demonstrated that these cells expressed COX-2 [19]. These authors also demonstrated a profoundly decreased antibody production in response to COX-inhibiting drugs. This is in line with our new observation that diclofenac-treated GD patients show reduced concentrations of anti-TPO. However, the TRAb concentrations were found to be relatively unchanged in response to diclofenac and therefore other mechanisms besides COX-2-mediated processes must exist to explain the lack of effects on TRAb concentrations. This is supported by an observation in COX-2 knockout mice where the concentrations of IgM and IgG were reduced by 64 and 35%, respectively, or less as compared to normal controls [19]. We have previously shown that COX-2 is upregulated in orbital tissue of patients with active GO and decreased in the chronic phase [12, 14]. COX-2 is an immediate early gene upregulated in response to mitogens, and among this group of genes, regulating inflammation and adipogenesis, CYR61 and EGR-1 have been found to be strongly overexpressed in patients with GO.

It was later shown that skin fibroblasts from thyroid healthy individuals express both these genes in response to cigarette smoking [20]. We have recently demonstrated that several immediate early genes including CYR61, EGR1 and COX-2 are overexpressed in orbital tissue of smokers with severe GO as compared to nonsmokers [21]. Tobacco smoking is a risk factor for development of both GD and GO, and it was stated in a recent review that current smoking increases the risk of GD approximately twofold and GO approximately threefold [3]. In our study we found that the proportion of smokers in the group with TRAb ≤ 2 IU/l was lower and that the TRAb concentration was higher after 15 months of treatment with antithyroid drugs and L-thyroxine as compared to nonsmokers. This is in contrast to a previous study demonstrating that thyroid-stimulating immunoglobulins are not influenced by smoking, which may be explained by the lower sensitivity of TRAb assay at that time [22]. However, in a recent study in GD patients treated with carbimazole, the presence of stimulating TRAb showed a much slower reduction in smokers than in nonsmokers [23]. In another autoimmune disease, rheumatoid arthritis, the number of patients with elevated levels of anti-

CCP was increased in smokers [24]. These authors suggested that patients with the genotype HLA-DR shared epitope are more susceptible to triggering immune reactions to citrullinated proteins in response to smoking. HLA-DR is a well-known risk genotype for development of GD, but GO patients lacked measurable concentrations of anti-CCP in smokers and nonsmokers [25]. However, some mechanisms may be shared since the CYR61 gene has been found to be overexpressed in peripheral blood of lymphoblastoid B cell lines from disease discordant monozygotic twins with RA [26]. The CYR61 gene was one of the most abundantly overexpressed genes in patients with severe GO in a previous microarray study [14]. Whether CYR61 is regulated by smoking in GD patients is still an open question, but in a recent study it was shown that an SNP in CYR61 increases the risk to develop GO more than fourfold in smokers [27].

To conclude, treatment with diclofenac had no significant influence on development of GO. Diclofenac seems to be safe to use in GD patients and reduces anti-TPO titers, but not TRAb titers. In addition, smokers have increased titers of TRAb, which may result in prolonged treatment of GD.

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Disclosure Statement

The authors have nothing to declare.

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