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ORIGINAL COMMUNICATION

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The combination of cyclophosphamide plus interferon beta as rescue therapy could be used to treat relapsing-remitting multiple sclerosis patients

Twenty-four months follow-up

Abstract The aim of the present study was to evaluate the efficacy of the combination of cyclophosphamide (CTX) and interferon beta (IFN β) in a group of relapsing remitting (RR) multiple sclerosis (MS) patients who experienced treatment failure during IFN β therapy. It is the general experience that immunomodulatory agents (IMA) are only partially effective in RR patients. Recent data on the efficacy of immunosuppressive therapies for these patients are encouraging. The anti-inflammatory and immunosuppressive effects of CTX have been utilized to treat selected cases of multiple sclerosis with a progressive and worsening course as rescue therapy. Thirty RR MS patients with clinically defined MS who experienced treatment failure during IFN β therapy (2 or more relapses per year or 1.5 EDSS point worsening in one year) were enrolled in the study and treated with CTX iv pulse therapy added to IFN β and followed up for 24 months. As primary endpoints we evaluated the yearly relapse rate. We also evaluated the percentage of patients free of relapses and of EDSS variations. We analysed the results at one year before entry (T0: IFN β alone), 12 (T1) and 24 (T2) months after entry. Brain MRI was performed at T0, at T1 and T2. The 30 RR patients who had experienced a high number of relapses (rr = 1.4) at T0 showed a significant improvement in yearly relapse rate (rr = 0.4) at T1 and a further improvement (rr = 0.17) at T2 ($p < 0.001$). The percentage of patients free of relapse was 70 % at T2 ($p < 0.0001$). EDSS score changed from 2.6 ± 1.23 at T0 to 2.2 ± 1.5 at T2, showing only a trend of improvement. No significant variation of MRI lesion load and no severe adverse events were recorded during the study. These data showed that the combination of CTX plus IFN β halted the progression of disease in active and deteriorating MS patients suggesting the necessity of RCTs to test the efficacy of this combination therapy in active RRMS patients or in patients who experienced treatment failure in response to disease modifying drugs (DMDs).

Key words multiple sclerosis · relapsing remitting · combination therapy · cyclophosphamide

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Introduction

MS is an inflammatory cell-mediated autoimmune disease affecting the central nervous system (CNS) [7, 24]. Cyclophosphamide (CTX) is an alkylating agent related to nitrogen mustards used in treatment of neoplastic and non-neoplastic immune-mediated inflammatory diseases [19, 20]. Its anti-inflammatory and immunosuppressive effects have been utilized to treat selected cases of multiple sclerosis with progressive and worsening course without obtaining a general agreement on the efficacy of this drug in modifying the course of the disease [1, 4, 5, 6, 8, 9, 14]. However literature data suggest that CTX is efficacious in MS when inflammation predominates over the degenerative process in the CNS and

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it is active on relapses and on gadolinium enhancing lesions on MRI. Moreover CTX has been shown to be active in the animal model of MS, experimental allergic encephalomyelitis (EAE) [15, 18, 26].

Although it has been shown that beta interferon (IFN β) or glatiramer acetate positively modify the natural course of MS [10, 12, 13, 33], it is the general experience that these immunomodulatory agents (DMDs) are only partially effective on relapsing remitting (RR) and have almost no effect on secondary progressive (SP) patients. This has encouraged the use of immunosuppressant agents such as mitoxantrone which is the only proven drug indicated for those MS patients who do not respond to DMDs and in those with the rapidly progressive form of disease [2]. With the need to modify the progression of disease in refractory patients, several open-label studies have recently shown positive results treating these patients with another immunosuppressant agent, CTX, following different treatments [4, 14, 23, 31, 39, 41]. In previous studies we demonstrated the effectiveness of a combination of IV monthly pulses of CTX and IFN β in patients with transitional MS, characterized by severe and frequent attacks and rapid progression of disability [29]. On the basis of the results obtained with the combination therapy on this group of patients and after the analysis of follow-up data which showed the maintenance of the benefits after 54 months [30], we decided to enlarge the combination treatment to those MS patients who did not seem to obtain any benefit from treatment with IFN β. The aim of the present study was to evaluate the efficacy and the safety of the combination treatment in a group of active RR MS patients previously treated with INF β.

Subjects and methods

Subjects

Eligible patients were relapsing remitting (RR) MS patients aged between 18 and 50 years, who fulfilled both Poser and McDonald diagnostic criteria [25, 32] and who had a baseline EDSS score between 2.0 and 6.0.

All patients included were eligible for IFN β treatment (two relapses in the preceding two years) who had been treated with IFN β for more than 12 months. Patients were eligible only in case of treatment failure with IFN β if they had experienced two or more relapses in one year or a severe relapse (worsening of > 2 points in any FS or 1.5 on EDSS score). Relapse was defined as symptoms of neurological dysfunction with objective confirmation, lasting at least 48 hours, following a period of symptomatic stability of at least 30 days, occurring in the absence of a febrile illness or steroid withdrawal and within 15 days of onset, showing an increase of at least 0.5 points on EDSS.

Patients were excluded if they were affected by liver, kidney, lung, cardiac failures, infections, blood diseases, other neurological and psychiatric diseases and pregnancy.

Patients were also considered not eligible if they had received immunosuppressive or immunomodulating treatments other than IFN β or methylprednisolone in the previous six months.

Endpoints

As primary outcome measures we considered relapse rate. We also evaluated percentage of patients free of relapses and EDSS changes during the observational period.

Brain MRI T2 lesion load and T1 Gd+enhancing lesions were measured. Safety and tolerability were also evaluated.

Study design and procedures

Thirty eligible RR MS patients who experienced treatment failure with INF β alone during the last 12 months of treatment and who accepted entry in the study (giving a written informed consent) received combination therapy with INF β and CTX. These patients were followed up during the 24 months of treatment.

CTX was administered in a IV monthly pulses regimen at a dose ranging from 500 mg/m^2 to 1500 mg/m^2 in order to obtain a chronic reduction of lymphocytes ranging from 600 mm^3 to 900 mm^3 at nadir as previously established [29]. Patients continued to receive standard therapeutic regimen of INF β (Avonex at the dose of 6 MIU once a week i. m., Betaferon 8 MIU every other day s.c or Rebif 44/22 μg 3 times a week s. c.) in combination with CTX.

Symptomatic treatment

Ondansetron was administered at the dose of 8 mg immediately before CTX administration. Large volumes of fluids and Mesna were administered e. v. on the day of CTX treatment to prevent bladder toxicity. Patients were also encouraged to drink large quantities of water in the three days following CTX administration.

IV methylprednisolone (1 g daily for 5 consecutive days) was permitted for the treatment of acute exacerbations.

Ethics

The local ethic Committee approved the study. Each patient gave written informed consent to entry in the study.

Outcome assessment

All patients underwent routinely complete physical and neurological examinations with EDSS determinations every 3 months. EDSS was assessed by an examiner different from the treatment giver.

Brain MRI was performed every 12 months from the beginning of combination therapy.

MRI lesion load was obtained with the Ormerod method [27] and gadolinium-enhanced lesions were recorded as well. MRI was carried out with a fast spin-echo GE 1.5-T Sigma Scanner, using TR = 2500 ms, TE = 18 ms/90 ms. Serial scans were obtained in the traverse plane from the level of foramen magnum to the vertex. The same positioning and imaging sequences were used throughout.

To evaluate safety and tolerability adverse events, vital signs, blood test and urine analysis were obtained every month; cytological analysis of urine was performed every 3 months. ECG, chest radiography, echography of liver, spleen, kidney, bladder, uterus and mammography were monitored at inclusion and at 12 months intervals thereafter.

Statistical Analysis

For the treatment group we compared the efficacy outcomes recorded during the 24 months of the combination therapy with data obtained during the previous 12 months (IFN β treatment alone).

The chi-square test, or Fischer's exact test when necessary, for dichotomous outcomes and parametric (t-test for paired data, analysis of variance) or non-parametric tests (Wilcoxon signed rank test) for continuous outcomes according to the distribution of variables were used to test significance. P value < 0.05 was considered statistically significant.

Annualized relapse rate was estimated for the 12 months preced-

ing the combination therapy and for the 24 months of follow up. Rate ratio and their relative 95% confidence interval (95% CI) were also calculated.

The percentage of patients free of relapse for the 12 months before and the 24 after the combination therapy was also evaluated.

Statistical analysis was performed using the software STATA (STATA Corporation. STATA release 6.0. College station, TX: STATA corporation 1993).

Results

In total 30 RR MS patients (23 women and 7 men) who experienced treatment failure with IFN β were consecutively enrolled when inclusion criteria were fulfilled. Twenty-six patients had been treated with IFN β 1a (16 with Avonex, 9 with Rebif 44 and 1 with Rebif 22) and 4 patients with IFN β 1b (Betaseron). All patients were put on combination therapy with CTX and IFN β. Baseline characteristics are shown in Table 1.

Combination therapy with IFN β and CTX was safe and well tolerated. During the 24 months of follow up 22 patients experienced transient and not severe headache after CTX administration lasting from 2 to 7 days. Twenty patients complained of nausea. None of them needed specific medications. Only 4 women of the 23 enrolled had amenorrhea; urinary tract infection was noted in 5 patients; only one patient experienced hemorrhagic cystitis. At cytological examination no patient exhibited cell alterations. No patient experienced scalp alopecia.

Concerning the efficacy endpoints as shown in Fig. 1 the relapse rate decreased from 1.4 observed during the 12 months before entry in combination therapy (T0: IFN β alone) to 0.4 during the first 12 months of combination therapy (T1) and 0.17 during the second 12 months (T2); considering the overall 24 months of follow-up period the annualized relapse rate was 0.28 (T1–T2) giving a rate ratio versus the 12 months of INF β alone of 0.20 (95% CI 0.10–0.35), demonstrating a dramatic decrease of the number of relapses after the beginning of combination therapy. None of the 30 patients were free of relapse during the 12 months of IFN β treatment (T0), while after 24 months of combination therapy 21 patients (70%) were free of relapse (T1–T2) (Fig. 2).

No significant changes in EDSS score have been recorded during the 24 months study period even if a trend of improvement was noticed. EDSS score changed from 2.6 ± 1.23 at T0 to 2.2 ± 1.5 at T2 (Fig. 3).

On MRI analysis T2 lesion load was respectively 38.2 cm^2 at T0, 39.3 cm^2 at T1 and 37.7 cm^2 at T2 showing no significant changes. The number of Gd+enhancing lesions decreased from 58 (T0) to 18 (T1) and only 3 at T2 ($p < 0.0001$).

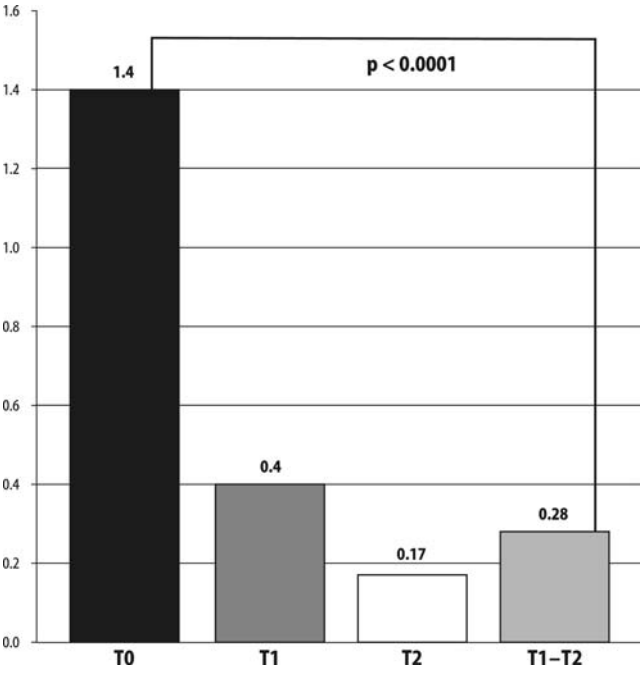

Fig. 1 Relapse Rate. *T0:* treatment with IFN B alone (12 months before entry); *T1:* treatment with CTX + IFN B (0–12 months); *T2:* treatment with CTX + IFN B (12–24 months); *T1–T2:* treatment with CTX + IFN B (total 24 months)

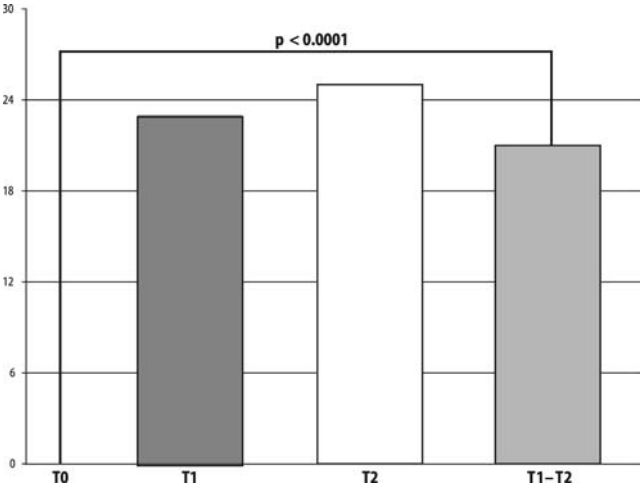

Fig. 2 Patients free of Relapse. *T0:* treatment with IFN B alone (12 months before entry); *T1:* treatment with CTX + IFN B (0–12 months); *T2:* treatment with CTX + IFN B (12–24 months); *T1–T2:* treatment with CTX + IFN B (total 24 months)

Table 1 Baseline characteristics

Sex	23 F/7M
Age at onset	25 ± 8yrs (median 24)
Age at entry	33 ± 6 yrs (median 33)
Disease duration	8 ± 4 yrs (median 7)
EDSS at entry	2.6 ± 1.23 (median 2.3)
IFN beta treatment duration before study entry	60 ± 23 mons (median 57)

F female; *M* male; *Yrs* years; *Mons* months

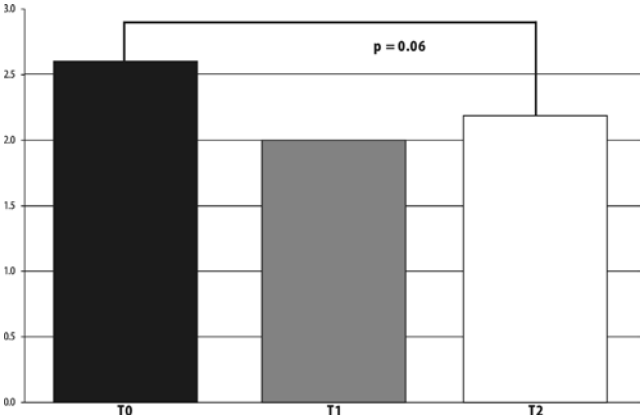

Fig. 3 EDSS. *T0:* treatment with IFN B alone (12 months before entry); *T1:* treatment with CTX + IFN B (0–12 months); *T2:* treatment with CTX + IFN B (12–24 months); *T1–T2:* treatment with CTX + IFN B (total 24 months)

Discussion

Several therapeutic clinical trials have been carried out in recent years with the aim of preventing and reducing the frequency and the severity of relapses and to slow the accumulation of disability in MS patients. The clinical effectiveness of IFN β for the treatment of MS has been documented by three double-blinded trials [10, 12, 33], as well as several smaller studies. Today these drugs represent the primary alternative in the treatment of RR MS; also in SP MS patients the use of IFN-β 1b has recently demonstrated some therapeutic effects [11]. Therefore it is well established that these therapeutic agents are able to modify the course of MS, but with only a total 30 % decrease of relapse rate in RR treated patients compared with placebo. The limited clinical efficacy of these drugs has led to the proposal of experimental alternative treatments in MS. Several immunosuppressive drugs have been tried in recent decades to treat those MS patients who did not seem to obtain clinical benefits by DMDs or with marginal efficacy. Mitoxantrone is so far the only proven immunosuppressant drug with an indication to treat MS patients who do not respond to DMDs or with active forms of disease [2]. As for other autoimmune diseases, the rationale for using immunosuppressive therapeutic strategies to control MS activity was to reduce the proliferation of autoreactive lymphocytes [3, 16, 17, 22, 37]; however most of the immunosuppressive treatments have shown only moderate efficacy in halting the progression of the disease. In addition they are usually associated with cumulative and toxic side-effects. Among the immunosuppressive drugs CTX has been tested, alone or in combination therapy, in several studies [34, 36, 40, 41]. Gonsette et al. reported the clinical experience of 141 MS patients treated with intensive iv. CTX immunotherapy, concluding that intensive immunosuppression is able to stabilise the disease and interfere with the pathological process involved in the pathogenesis of MS [5]. Hommes et al. treated 32 MS patients with CTX producing immunosuppression: benefits were shown especially in patients with shorter disease duration [9]. Hauser et al. reported evidence that progressive MS could be stabilised by short-term, intensive immunosuppression with CTX plus ACTH [8]. The Kaiser-Permanente Medical Care Program designed to evaluate the effects of intensive immunosuppression in MS showed modest benefits to the treatment but demonstrated the safety of outpatient CTX administration [21]. Carter et al. showed that high-dose iv CTX and ACTH treatment regimen was well tolerated and favourably affected the course of chronic progressive MS, but some forms of maintenance treatment or re-treatment were requested for persistent stabilisation of disease [1]. The North-Eastern Cooperative Treatment Group reported a benefit from CTX boosters, but the positive clinical effects disappeared in 36 months. Age was the most important variable which correlated with response to treatment: CTX boosters had a significant benefit in patients aged from 18 to 40 [38]. By contrast the Canadian Cooperative Multiple Sclerosis Study failed to demonstrate any beneficial effect of CTX on disease progression rate [35]. On the basis of this evidence the use of CTX for the treatment of MS has remained controversial. More recently, several open-label studies have shown positive results treating these patients with CTX following different treatments [29, 39, 40]. On the basis of the published data it is possible to state that IFN β effectively reduces the rate of exacerbation and the development of new MRI lesions, although its action is limited with only partial efficacy in RR and almost no effect in SP patients. It has also been demonstrated that IFN-β can be safely used for long periods. On the contrary CTX seems to be effective in arresting the inflammatory process, but its toxicity limits the treatment duration. Although CTX has been found to be well tolerated at the doses used in the available studies, its long-term use could be limited because of bladder toxicity and breast cancer and other side-effects. Toxicity is dose related and develops for cumulative dose above 75–80 g in three years. However, because of its powerful and rapid action CTX could be a good candidate for both induction and combining therapeutic agents. The use of induction or combining treatment has been demonstrated to be effective in infectious and neoplastic diseases, but it has been poorly investigated in MS. Since both IFN β and CTX have proven efficacy, sequential administration or combination of IFN β and CTX could represent a therapeutic strategy to treat those MS patients who do not respond adequately to DMDs. The specific and complementary effects of these two drugs in terms of immunosuppressive activity (global for CTX and selective for IFN β), and of rapid action (strong and immediate for CTX, progressive im-

munomodulation for IFN β) suggest that these two drugs if combined might exhibit synergetic action in MS.

In our study the use of CTX combined with IFN β maintained for 24 months in selected RR MS patients after treatment failure with IFN-β showed significant decrease of relapse rate.

Furthermore this therapeutical strategy increased the percentage of patients free of relapses (70%) and produced a stabilization of disease in terms of disability (EDSS) and new lesions on MRI. Indeed we observed that EDSS decreased from 2.6 to 2.2; although a two-year-period follow-up is too short to evaluate the progression of the disease in clinical practice, we did not observe in our patients the expected EDSS increase according to the natural history of MS. Likely MRI stability in terms of new T2 lesions seems to demonstrate that the combination therapy may act by slowing the progression of the disease with a main effect on the inflammatory components of MS. Also the reduction of the number of Gd-enhancing lesions at MRI confirms a decrease of inflammation. This evidence encourages us towards renewed interest in the possible benefit of a treatment with CTX in multiple sclerosis. As suggested by the literature, we obtained positive results administering iv CTX in intermittent pulse therapy regimen, in order to obtain an intensive immunosuppression [38]; another important topic that we considered in the selection of our patients in this open study had been already highlighted by previous clinical experiences with CTX: young patients (aged less than 40 years), with a relative short disease duration (less than 9 years) and with clinical and paraclinical (MRI) evidence of disease activity and CNS inflammation are the best candidates for this kind of treatment (for more details see baseline characteristics in Table 1). At the same time the review of the literature suggests that the efficacy of CTX is short-lasting and that the benefits obtained with the strong immunosuppression induced by CTX disappear with a re-progression of disease. For this reason we have used CTX as add-on therapy, in combination with IFN β whose immunomodulatory action is well known. This combination regimen whose rationale is based on the synergic action of the two drugs could also limit the use of high amounts of CTX reducing the risk of adverse side effects and inducing a long-lasting immunosuppression. A treatment with pulse therapy of CTX lasting 24 months represents in our opinion a good period in which to obtain strong immunosuppression giving an amount of drug significantly less than the dangerous cumulative dose above 80 g and allowing the possibility of retreating if there is re-progression of disease. In our experience the first six months of treatment with combination regimen were important to establish the individualized dose of CTX in order to obtain a chronic reduction of lymphocytes count at nadir, which is the basical aim of this treatment. In any case the distribution of the number of relapses during the total period of observation showed the dramatic decrease in the number of relapses after the beginning of the combination, demonstrating the strong and rapid action of CTX as an anti-inflammatory drug. Our results showed how during the second twelve months of treatment (T2) there was a further reduction of relapse rate (from 0.4 at T1 to 0.17 at T2) indicating a further decrease of disease activity during the second year of treatment (see Fig. 1).

The necessity of an early and strong therapeutic action against the inflammation process which leads to demyelination and progressive axonal loss in the CNS during MS and the limited results obtained treating MS patients with DMDs alone, suggested to us that we should define a therapeutic schedule using CTX and IFN β in combination therapy in those patients with active and worsening forms of disease as rescue therapy who did not seem to benefit from IFN β treatment alone.

The results obtained in this open trial even with the limits due to design of the study suggest a possible efficacy of the combined regimen of CTX and IFN β. This study adds new useful information for the treatment of RR MS patients who failed to IFN β therapy. Differently from our previous studies the population of patients here investigated were RR with an active form of disease, and poor responders to IFN β treatment. This sample could be more representative of RR MS population than the rapidly transitional groups previously described [29, 30]. Thus the present results could suggest a therapeutic option for RR MS in the earlier stages of the disease when inflammatory activity is prominent. It is our intention to follow-up these 30 RR patients treated with combination therapy after the discontinuation of CTX when they will be treated again with IFN β alone, but we think that further controlled and randomized studies are necessary to define the role that this combination regimen could play in the treatment of MS [28].

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