Combination therapy with mitoxantrone and plasma exchange in aggressive relapsing remitting multiple sclerosis: A preliminary clinical study

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Background: The efficacy of mitoxantrone induction therapy in rapidly worsening multiple sclerosis (MS) is well established. Plasma exchange is also applied as an adjuvant in exacerbations of relapsing MS. The aim of this study was to compare the efficacy of combination therapy with mitoxantrone and plasma exchange versus mitoxantrone alone in patients with aggressive MS. **Materials and Methods:** Forty patients with aggressive relapsing remitting MS were randomly put into two groups. The first group underwent monthly plasma exchange for three successive months, followed by 12 mg/m² mitoxantrone at the end of each course and two more doses of 6 mg/m² mitoxantrone in 3-month intervals. The second group received the same doses of mitoxantrone only without plasma exchange. At the end of 8 months treatment course, clinical reassessment and neuroimaging was performed and treatment was continued with interferon-β. **Results:** At the end of induction therapy, Expanded Disability Status Scale score was significantly improved in both groups ($P < 0.001$). Number of demyelinating and gadolinium-enhancing plaques in brain magnetic resonance imaging (MRI) was prominently reduced in group 2 ($P \le 0.05$), but the changes were not statistically significant in group 1, except for juxtacortical plaques. **Conclusion:** Administration of mitoxantrone as an induction therapy in patients of aggressive relapsing remitting MS results in significant improvement of their clinical state and MRI activity. However, combination of plasma exchange with mitoxantrone gives no more benefits than mitoxantrone alone and sometimes worsens the situation possibly by reduction of mitoxantrone efficacy as a result of plasma exchange.

Key words: Induction therapy, mitoxantrone, multiple sclerosis, plasma exchange

INTRODUCTION

Multiple sclerosis (MS) is the most common demyelinating disease of central nervous system (CNS). It is also the leading cause of disability among the youth. Control of disease activity in rapidly worsening or aggressive MS is a great challenge for clinicians. Although there are different definitions for aggressive MS in the literature, it can be best defined as occurrence of at least two relapses with sequel or an increase of at least two points in Expanded Disability Status Scale (EDSS) score during the last 12 months with the presence of at least one gadolinium (Gd)-enhancing lesion on the patient's magnetic resonance imaging (MRI) performed within the last 3 months.[1]

Although treatment with first-line disease-modifying drugs is proved to reduce the number of relapses and slow down disability progression, they have varying degrees of efficacy on disease control in patients with aggressive MS. Recent evidences suggest that treatment outcome may be improved in such patients by adding various agents to their treatment protocol as combination therapy. One of such combinations for patients at high risk of disability progression is induction therapy with a cytotoxic agent followed by either switching to or adding a disease-modifying drug such as interferon-β (IFN-β) or glatiramer acetate.[2-7]

Several studies have shown the efficacy of induction therapy with mitoxantrone on relapse rate, EDSS score, and MRI activity in patients with rapidly worsening MS.[2,3,8,9] Therefore, mitoxantrone is regarded as the choice among cytotoxic agents.

Some other studies assessed the effect of combining a cytotoxic agent such as azathioprine or cyclophosphamide with plasma exchange (PE).[10-13] PE is a modality that is now applied as an adjunctive in the treatment of exacerbations in relapsing forms of MS (Level B) and also in the treatment of fulminant CNS demyelinating diseases that fail to respond to high-dose corticosteroids (Level C).[14] Investigations on this type of combination therapy had controversial

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results. Some reported favorable effects, whereas others declared no benefits.[10-13]

No trial has so far been carried out to evaluate the effect of mitoxantrone plus PE on the aggressive forms of MS. This study was therefore designed to assess the efficacy of a novel combination therapy including PE and mitoxantrone in patients with aggressive MS.

MATERIALS AND METHODS

This study was approved by Isfahan University of Medical Sciences' ethics committee and all patients signed an informed letter of consent prior to their inclusion.

Patients

In this study, a total of 52 aggressive MS patients admitted to Isfahan MS Referral Clinic from December 2009 to July 2010 were enrolled and randomized into two groups alternately based on their visit date. They were also matched on demographic and clinical characteristics. Both groups consisted of clinically definite MS patients (revised Mc Donald criteria 2005),[15] aged between 18 and 55 years with EDSS score of 1–5. All of them were defined to have aggressive MS containing the criteria of at least two relapses with sequel or an increase of at least two points in EDSS score during the last 12 months with the presence of at least one Gd-enhancing lesion on the patient's MRI performed within the last 3 months.[1] None of them ever received mitoxantrone and had no contraindications for it.[5,9,16] They did not use corticosteroids or any other immunosuppressants in the past 3 months. All of the patients were under treatment with IFN-β before enrolling in our study. Five patients had received Avonex, 23 were on Rebif, and 12 were on Betaferon.

Baseline clinical and laboratory assessments and treatment protocol

At the first visit before treatment was started, an expert neurologist took a thorough history and performed physical examination on each patient. Past clinical attacks were confirmed and annual relapse rate (ARR) was determined for each one. Brain and spinal cord MRI with Gd was asked for every individual. Then, all patients were given intravenous (IV) methylprednisolone (Pharmacia company, Diegem, Belgium) 1000 mg daily for 5 days. Females were instructed to use an effective contraceptive method.

After 30 days of corticosteroid therapy, all patients were reevaluated by the same neurologist and their EDSS scores were recorded. Lab examinations including complete blood cell count (CBC), liver function tests (LFT), besides electrocardiography (ECG), chest X-ray (CXR), and echocardiography were requested for each patient. Patients with abnormal test results were excluded.

We started monthly PE (plasma exchange machine: Haemonetics, model TCS2, USA) 25 ml/kg for 5 cycles, with replacement of 0.9% saline and 5% human serum albumin for the first group, followed by monthly IV infusion of 12 mg/m^2 mitoxantrone (EBEWE Pharma, Amsterdam, The Netherlands) at the end of each PE course for three successive months. Then, we continued treatment by adding two more 6 mg/m^2 doses of mitoxantrone in 3-month intervals. The second group received the same doses of mitoxantrone only without PE. Except MRI, all patients had to have CBC, LFT, and echocardiography reports prior to each dose of mitoxantrone to check for any probable abnormalities. Treatment with IFN-β was started for both groups after the last dose of mitoxantrone.

Short-term and long-term follow-up

We planned to reevaluate the patients at two stages: first when induction therapy was completed (short-term follow-up) and then 9 months later (long-term follow-up). EDSS score and relapse rate were clinically assessed at both stages by the same neurologist blinded to patients' groups.

In case a new symptom developed, the patient was instructed to inform our clinic. Such patients were visited within 48 h from their call. A relapse was defined as new or worsening neurological symptoms or signs in the absence of fever, persisting for at least 24 h in a patient with a stable condition in the last month.

Secondary progression anytime during the study period led to exclusion of patient from the study and showed the need for a new treatment.

MRI protocol and analysis

Brain and spinal cord MRI was performed using a 1.5 T scanner at a single center including T1-weighted (with and without Gd) images, T2-weighted images, fluid-attenuated inversion recovery (FLAIR), and proton density sequences. Slices of 3-mm thickness were acquired for each sequence. Post-contrast T1-weighted imaging was performed 5 min after injection of 0.1 ml/kg Gd given over 30 sec.

All patients underwent brain and spinal cord MRI at two stages, along with follow-up clinical assessments. We decided to delay the follow-up MRI scan for 30 days if the patient experienced a relapse and was treated with corticosteroids to avoid the probable effect of steroids on MRI scan.

A neuroradiologist blinded to groups analyzed all MRI scans. Number of existing lesions in different anatomical locations and also active lesions (Gd-enhancing T1-weighted lesions) in post-treatment MRI (at the end of induction therapy) were compared with the baseline scan.

Outcome measures

The primary outcome was evaluation of the changes in EDSS score, relapse rate, and also MRI images (number and location of plaques, number of Gd-enhancing lesions, and black holes) at the end of induction therapy.

The secondary outcome included assessment of changes in the EDSS score and ARR from baseline to the end of the study, and absolute changes in brain and spinal cord MRI (number and location of plaques, number of Gd-enhancing lesions, and black holes) from baseline to month 17.

In this paper, we only considered the therapeutic effects of combination therapy on the primary outcome.

Statistical analysis

All data were analyzed on a computer using SPSS ver. 18 software package (SPSS Inc., Chicago, USA). Independent t-test was used to compare age, ARR, disease duration, baseline EDSS score, and number of MRI plaques of the two groups. We used chi-square test to compare sex and initial symptom and Fisher's exact test to analyze family history and relapses during treatment. Baseline and follow-up changes in EDSS and MRI findings were examined using paired t-test. Correlation between EDSS change and study variables was determined by Pearson correlation coefficient. Statistical significance was defined as P value ≤0.05.

Limitations

Although the neurologist who recorded EDSS scores and the neuroradiologist who reported MRI scans were masked to patients' data, we failed to design a double-blinded study because we could not apply a sham procedure for patients in group 2 who only received mitoxantrone.

RESULTS

A total of 52 patients who met the inclusion criteria were invited to participate in this study. Of these, 18 patients in group 1 and 22 in group 2 completed the study, while 12 patients were excluded or withdrew because of new plan for pregnancy, refusal to receive interferon during follow-up, or emigration.

Demographic and clinical characteristics

Demographic and clinical data of both groups were similar at baseline [Table 1].

Disease activity during the year before treatment

In each group, four patients with an increase of at least two points in EDSS score during the last year were included. They had at least one Gd-enhancing lesion on their recent MRIs.

Rest of the patients [14 (78%) in group 1 and 18 (81%) in group 2] had at least two relapses with sequel in the year before starting treatment (mean relapse rate was 2.4 and 2.2 for groups 1 and 2, respectively).

Short-term effect of treatment on clinical and MRI indices

EDSS values before and after treatment are presented in Table 2.

- EDSS: EDSS score decreased significantly at the end of treatment in both groups ($P < 0.001$). Yet, the difference was not significant between the two groups.

**Table 1: Demographic and clinical characteristics of patients**

Variable	Group 1[†]	Group 2	P-value
Gender			0.97
Female, *n* (%)	14 (77.8)	17 (77.3)	
Male, *n* (%)	4 (22.2)	5 (22.7)	
Age (years)*	30.4 ± 11.3	29.1 ± 6.7	0.65
Duration of disease (years)*	5.7 ± 3.2	4.6 ± 3.2	0.28
Initial symptom, *n* (%)			
Brainstem	5 (27.8)	5 (22.7)	
Pyramidal	10 (55.6)	7 (32.8)	
Optic neuritis	2 (11.1)	7 (31.8)	
Sensory	1 (5.6)	3 (13.6)	
ARR*	1.2 ± 0.7	1.3 ± 0.6	0.65
Family history of MS (%)	16.7	4.5	0.23

ARR, annual relapse rate [†]Group 1: Plasma exchange and mitoxantrone induction therapy; group 2: mitoxantrone induction therapy; *Values are expressed as mean ± SD

Table 2: EDSS response to treatment

	Baseline EDSS	Post-treatment EDSS[†]	P-value
Group 1	3.7 ± 1.2	2.9 ± 1.3	<0.001
Group 2	3.5 ± 1	2.6 ± 0.9	<0.001
P-value	0.62	0.4	

EDSS, expanded disability status scale [†]Post treatment EDSS: EDSS score at the end of induction therapy [‡]Group 1: Plasma exchange and mitoxantrone induction therapy, group 2: mitoxantrone induction therapy; Values are expressed as mean ± SD
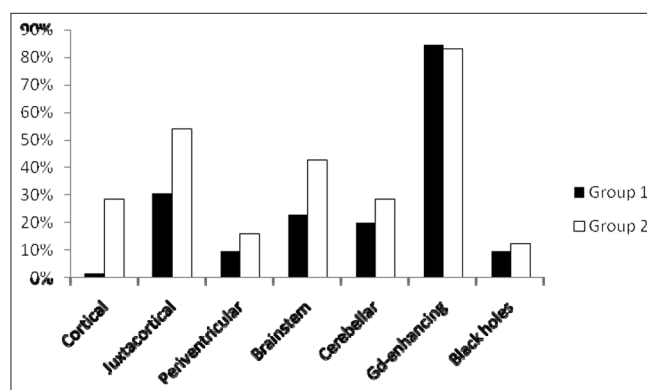
Figure 1: Comparison of reduction rate in demyelinating plaques of groups after treatment

- Relapses: One patient in group 1 experienced a clinically proved attack during induction therapy, 5 months after the treatment had started. Also, two patients in group 2 experienced attacks in the fourth and fifth months of the study. They all had to undergo methylprednisolone pulse therapy.
- MRI findings: Although treatment decreased the number of plaques in both groups, this decrease was not significant in group 1 except for juxtacortical plaques ($P < 0.05$) [Figure 1]. In contrast, the number of plaques in group 2 significantly reduced in all anatomical locations ($P < 0.05$). Reduction in the size and number of black holes in both groups and Gd-enhancing lesions in group 1 was not significant. [Table 3] Yet, the number of Gd-enhancing lesions significantly decreased in group 2 ($P = 0.05$) [Tables 3, 4].

Mean number of new T2 lesions was 1.9 ± 0.5 in group 1 and 1.1 ± 0.3 in group 2. There were no significant differences between the two groups ($P = 0.16$).

PREDICTORS OF RESPONSE TO TREATMENT

Prognostic factors

Reduction in EDSS score in the short-term follow-up had a reverse relation with age and disease duration ($P = 0.02$ and $P < 0.001$, respectively). However, there was a positive correlation between ARR and EDSS decrease ($P = 0.002$) since patients with higher ARR showed greater clinical response to treatment in both groups. Treatment results had no relation with sex, presenting symptom, or selecting criteria (increase in EDSS or relapses with sequels). Although EDSS decreased significantly in both groups, MRI activity suppression was only significant in group 2 patients, so most prominent results came from younger patients with shorter duration of disease and higher ARR, who were treated with mitoxantrone alone.

Safety assessment

Fortunately, no serious adverse conditions were reported in patients and no therapy withdrawal happened due to drug side effects or abnormal laboratory data. However, there were some minor side effects due to mitoxantrone administration, as expected. Mild anemia was observed in two patients. Transient rise in liver enzymes was detected in one patient. Nine females experienced transient amenorrhea and one patient had moderately intractable nausea after mitoxantrone infusion. No clinically symptomatic cardiac event was found in any of our patients. Similarly, no PE-related complications such as symptomatic hypocalcemia, hypotension, infusion site complications, or hemolysis were reported.

Table 3: Comparison of baseline and post-treatment MRI findings in group 1

Plaques	Group 1		Mean plaque reduction	*P*-value
	Plaque numbers			
	Baseline MRI	Post-treatment MRI*		
Gd-enhancing	1.3 ± 0.7	0.2	1.1	0.07
Black holes	8.3 ± 1.7	7.5 ± 1.4	0.8	0.16
Cortical	5.9 ± 1.7	5.8 ± 1.6	0.1	0.46
Juxtacortical	3.6 ± 0.9	2.5 ± 2	1.1	0.04
Periventricular and subcortical	17.3 ± 2.6	15.6 ± 2.5	1.7	0.1
Brainstem	1.3 ± 0.3	1 ± 0.3	0.3	0.09
Cerebellar	1 ± 0.4	0.8 ± 0.3	0.2	0.3
Total T2 plaque numbers	29.4 ± 1.2	25.9 ± 1	3.5	0.19

Gd; gadolinium *Post-treatment MRI: MRI at the end of induction therapy; Values are expressed as mean ± SE

Table 4: Comparison of baseline and post-treatment MRI findings in group 2

Plaques	Group 2		Mean plaque reduction	*P*-value
	Plaque numbers			
	Baseline MRI	Post-treatment MRI		
Gd-enhancing	0.6 ± 0.3	0.1	0.5	0.05
Black holes	8.1 ± 1.6	7.1 ± 1.6	1	0.08
Cortical	5.6 ± 0.8	4 ± 0.8	1.6	0.001
Juxtacortical	3.5 ± 0.8	1.6 ± 0.2	1.9	0.01
Periventricular and subcortical	18.1 ± 2.7	15.2 ± 2.4	2.9	0.01
Brainstem	2.1 ± 0.4	1.2 ± 0.2	0.9	0.01
Cerebellar	0.7 ± 0.3	0.5 ± 0.2	0.2	0.02
Total T2 plaque number	30.2 ± 1	22.9 ± 0.8	7.3	0.01

Gd; gadolinium *Post-treatment MRI: MRI at the end of induction therapy; Values are expressed as mean ± SE Support: There are no financial or other kinds of support from any company.

DISCUSSION

Therapeutic PE constitutes an extracorporeal blood purification technique designed to remove large molecular weight particles from plasma. The aim of the procedure is to deplete the blood of various immunological factors, such as circulating autoantibodies, immune complexes, cytokines, and other inflammatory mediators, which may be associated with disease pathology. Clearly, this is a short-term solution since antibodies are made by B cells that will continue to synthesize pathogenic autoantibodies in response to repeated antigenic stimulation after the PE is completed. So, the transitory effects of PE require the combination of short-term active PE with long-term immunosuppression. Also, the sudden elimination of feedback inhibition of B cells due to sudden decline in autoantibody levels may synchronize their activity and make them particularly sensitive to a pulse of immunosuppression.[17]

We designed this study based on the possible benefit of these immune mechanisms.

As shown in other studies,[2-4,8,9,18] the therapeutic effect of mitoxantrone induction therapy on our patients' clinical status was significantly evident. Patients' EDSS and relapse rate decreased during the first 8 months of treatment in both groups. On MRI images, however, patients who underwent PE showed less favorable response to treatment compared to those who received mitoxantrone only.

There are some probable mechanisms responsible for this result. First of all, we should consider that PE may result in an increased antibody production after the procedure. The study of Goldammer *et al.*[19] on the influence of plasma immunoglobulin level on antibody synthesis demonstrated that in almost all patients (88%) who underwent PE, the total immunoglobulin G (IgG) level reached pre-treatment values within 24 h of the procedure and the level remained within normal range during further course. The responsible mechanism is proved to be changes of catabolism and immunoglobulin backflow instead of increased antibody re-synthesis. It is probable that significant number of our patients also experienced increased autoantibody level after PE by a different mechanism that mitoxantrone had no effectiveness on it. Mitoxantrone inhibits B-cell function and antibody secretion, but has no effect on the rate of antibody catabolism or rapid antibody redistribution from extravascular to intravascular space. So, in this way, PE could reduce the strict therapeutic effect of mitoxantrone on lowering antibody levels.

Another possible mechanism was explained by the investigation of Horng Yeh *et al.* In this study of 18 healthy volunteers, a single session of membrane PE activates the cellular immunity in terms of increased B cells and higher T-helper/T-suppressor ratios.[20] This role of PE on immune system has a contrary effect on mitoxantrone function that exactly does the reverse.

All the studies on the effectiveness of PE in acute relapse of MS showed promising effects;[21-25] however, studies on combination therapy of an immunosuppressant (cyclophosphamide) and PE had controversial results. The short-term beneficial effect of combination therapy with adrenocorticotrophin hormone (ACTH), cyclophosphamide, and PE versus ACTH, cyclophosphamide, and sham exchange was reported by Weiner *et al.*[11] Similar results were reported by Khatri *et al.* in a randomized, controlled, double-blinded trial that investigated the efficacy of PE, methylprednisolone, and cyclophosphamide in comparison with methyl prednisolone, cyclophosphamide, and sham treatment among patients with a progressive form of MS, which showed clinical benefit after 5 months in the PE group compared to the sham treatment group.[12] In both these studies, PE was performed in longer intervals than in our survey. This protocol probably increases the amount of excluded antibody by PE as a result of enough time available to remove redistributed antibodies from extravascular space. The other prominent characteristic of these two studies was administration of cyclophosphamide and methylprednisolone/ACTH during the PE treatment period, which perhaps prevented the accumulation of unfavorable effects of PE such as increased B-cell and T-helper numbers.

A larger trial from the Canadian Cooperative Multiple Sclerosis Study Group, which randomized patients with chronic progressive MS to receive daily intravenous (IV) cyclophosphamide and oral prednisone, daily oral cyclophosphamide and oral prednisone in alternate days and weekly PE, or placebo and sham PE, showed no improvement in the EDSS in 6 and 12 months of follow-up. The precise effect of PE cannot be determined in this study because of different administration protocol of cyclophosphamide and prednisone in the study arms.[10]

The efficacy of PE either could not be revealed in Hauser *et al.*'s study which compared a short course of high-dose IV cyclophosphamide and ACTH (caused 80% disease stabilization) with ACTH alone (20% disease stabilization) and with low-dose cyclophosphamide with ACTH and PE (50% disease stabilization) because of the prominent effect of high-dose cyclophosphamide in comparison to its low dose in PE group.[26] None of these studies investigated the effect of combination therapy on MRI activity.

In conclusion, although PE is useful in the treatment of corticosteroid-unresponsive MS attacks based on the suggested role of humoral factors in the pathogenesis of MS, it

seems that its benefit on aggressive form of MS needs special treatment protocol that at least includes longer intervals between PE courses to increase the efficacy and decrease the adverse effects of compacted exchanges on immune system and also co-administration of immunosuppressive drug during the PE courses to reduce the enhancing effect on B-cell and T-helpers. However, more trials are needed to determine the exact role of PE and the desired number or volume of exchanges to achieve the best therapeutic response. The long-term effect of PE on mitoxantrone induction therapy remains to be determined in our patients.

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