Efficacy and Safety of Mitoxantrone in Patients With Highly Relapsing Neuromyelitis Optica

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Objective: To evaluate the efficacy and safety of mitoxantrone hydrochloride and determine how it exhibits a differential inhibitory effect on subsets of B cells in patients with highly relapsing neuromyelitis optica (NMO).

Design: Retrospective case series with prospective follow-up.

Setting: Three referral medical centers in the Republic of Korea.

Patients: Twenty patients with highly relapsing NMO or neuromyelitis optica spectrum disorder who had at least 2 relapses during the year preceding the start of mitoxantrone treatment, despite other immunotherapies.

Intervention: Infusions of mitoxantrone up to a maximum cumulative dose of 120 mg/m².

Main Outcome Measures: Annualized relapse rate, disability according to the Expanded Disability Status Scale score, and fraction of CD27⁺CD19⁺ memory B cells.

Results: During mitoxantrone treatment, the median annualized relapse rate was reduced by 75%, and 50% of patients became relapse-free. Disability improved or stabilized in all patients. No patients had serious adverse effects during the mean follow-up period of 41 months after completing therapy. Flow cytometric analysis of cell surface markers revealed that mitoxantrone treatment preferentially affected CD27⁺CD19⁺ memory B cells.

Conclusions: Treatment with mitoxantrone in patients with highly relapsing NMO significantly reduces relapse rates, resulting in subsequent functional stabilization or improvement.


Neuromyelitis optica (NMO) is a rare inflammatory disease of the central nervous system that is characterized by optic neuritis and longitudinally extensive transverse myelitis. The discovery of a specific autoantibody against aquaporin 4 in patients with NMO has led to a marked increase in the understanding of the autoimmune mechanisms of the disease and suggests a role for B cell–mediated autoimmunity. Up to 80% of patients with typical NMO have NMO IgG or anti-aquaporin 4 antibodies in their serum, and this antibody is implicated in the disease’s pathogenesis. In addition, the low efficacy of interferon beta in the prevention of NMO relapses and the upregulation of B cell–activating factor in patients with NMO supports the role of B cell autoimmunity.

The disabilities associated with NMO are usually the result of poor recovery from relapses; therefore, effective treatment to prevent the occurrence of relapses is required. Several studies have described the benefits of immunosuppressant agents in the prevention of NMO relapses. Nevertheless, therapeutic experience with NMO is limited to either small or retrospective case series, and high-quality evidence is still insufficient to support a role for immunosuppressive treatment.

Mitoxantrone hydrochloride is currently the only cytotoxic agent approved for the treatment of worsening relapse-remitting, secondary progressive, and relapsing progressive multiple sclerosis (MS). Although mitoxantrone causes generalized immunosuppression, recent studies have reported that treatment with this drug induces a differential inhibitory effect on subgroups of leukocytes and preferentially targets CD19⁺ B cells. Considering the preferential suppression of humoral immunity by mitoxantrone, it is likely an effective treatment for NMO, as humoral immunity plays a pivotal role in its pathogenesis. However, there is little...
evidence of the effectiveness of mitoxantrone treatment in NMO, especially highly relapsing NMO.

This study evaluated the effectiveness and safety of mitoxantrone treatment based on experience with Korean patients with NMO who had frequent relapses despite other immunomodulating and/or immunosuppressive therapies. The differential inhibitory effect on a subgroup of B cells following mitoxantrone treatment was also investigated.

METHODS

PATIENTS

We recruited consecutive patients with relapsing NMO (using diagnostic criteria from 2006) or NMO spectrum disorder who were treated with mitoxantrone between July 2005 and October 2008. All patients presented with at least 2 relapses during the 12 months preceding the start of mitoxantrone therapy, despite immunotherapies using interferon beta, azathioprine, prednisolone, cyclophosphamide, or a combination of these drugs. Patients with cardiac dysfunction, hepatic or renal disease, abnormal baseline white blood cell or platelet counts, pregnant or lactating women, and those of reproductive age who were not willing to use contraception were excluded from mitoxantrone therapy. The study was approved by the institutional review board of National Cancer Center, and informed consent was obtained from all patients.

TREATMENT

Initially, 7 patients were treated with 3 monthly cycles of 12 mg/m² of intravenous mitoxantrone infusions followed by 6- to 12-mg/m² infusions every 3 months for maintenance. Patients received a maximum dose of 100 to 120 mg/m². However, 3 of 7 patients still experienced relapses during the maintenance period when the infusion interval was lengthened to 3 months. Based on our experience with these initial patients, the treatment protocol was changed to 6 cycles of 12 mg/m² monthly intravenous infusions as an induction, followed by maintenance treatment of 6 to 12 mg/m² every 3 months up to a maximum dose of 100 to 120 mg/m². The remaining 13 patients were treated with the changed protocol.

CLINICAL DATA ASSESSMENT

Clinical data were obtained through a retrospective review of medical records. The number of relapses and Expanded Disability Status Scale (EDSS) scores were used as parameters of effectiveness and were compared between premitoxantrone and postmitoxantrone treatment during a mean follow-up period of 17 months (range, 8-22 months).

The patients were monitored for adverse events during mitoxantrone treatment. Hemoglobin level, white blood cell and platelet counts, and serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase levels were evaluated prior to and 10 days after each infusion. Cardiac function was monitored by 2-dimensional echocardiograms at baseline and before every other mitoxantrone administration. All patients were followed up and continued treatment with other drugs (data not shown) until May 2010, and they were evaluated for the development of delayed adverse effects, especially therapy-related acute leukemia, during a mean period of 41 months. Attacks were defined as objective worsening of new neurological symptoms lasting at least 24 hours that increased the EDSS score by at least half a step (0.5), increased scores on 2 different functional systems of the EDSS by 1 point, or increased the score on 1 functional system (excluding bowel/bladder or cerebral functional systems) by 2 points.

FLOW CYTOMETRIC ANALYSIS OF CELL SURFACE MARKERS

After obtaining informed consent, samples were collected from 10 of 20 patients with NMO at baseline and after completion of 6 cycles of monthly mitoxantrone infusions. We used simple whole-blood staining for characterization of the leukocytes and B-cell subsets directly out of the circulation. This approach minimizes potential changes in levels of activation markers that may be induced in the cells of interest through additional in vitro processing. Triple-color immunofluorescent staining of whole-blood samples were performed within 60 minutes of blood drawing using antibodies against CD14/CD3/CD19 and CD27/CD19 with isotype controls, followed by lysis of red blood cells and immediate acquisition and analysis by flow cytometry.

STATISTICS

The EDSS scores, annualized relapse rates, and proportion of leukocyte subsets and CD27/CD19 B cells were compared before and after mitoxantrone treatment using the Wilcoxon signed rank test. All statistical analyses were performed using GraphPad PRISMS (San Diego, California), and P < .05 was considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS

Twenty patients with aggressive NMO (n = 16) or NMO spectrum disorder (n = 4) were included in the study. All patients were female, and results of laboratory testing were seropositive for anti–aquaporin 4 antibody. The average age for starting to receive mitoxantrone therapy was 34.3 years (range, 20-58 years), and the median interval from the onset of NMO or NMO spectrum disorder to treatment with mitoxantrone was 6.1 years (range, 1.1-13.3). During the year before mitoxantrone treatment, the 20 patients presented with a mean of 4 relapses (range, 2-7). The clinical and demographic profiles of the patients are outlined in the Table.

TREATMENT EFFICACY

Figure 1 illustrates how the relapse frequency markedly declined after mitoxantrone treatment. The median pretreatment annualized relapse rate was 2.8 (range, 1.0-5.7), and the median posttreatment annualized relapse rate was 0.7 (range, 0-2.3; P < .001). Half of the patients became attack free during the average mitoxantrone treatment period of 17 months. A total of 18 relapses occurred during mitoxantrone treatment. Of them, 7 occurred in 3 of the patients who were initially treated with 3 cycles of monthly mitoxantrone. Relapses in the rest of the 13 patients who were initially treated with 6 cycles of monthly mitoxantrone primarily occurred in the early stages of treatment. Only 1 relapse was noted after completion of 6 cycles of monthly mitoxantrone infusion. The posttreatment EDSS scores of the patients were significantly better than pre-
Flow cytometric analysis of cell surface markers revealed significant changes in a proportion of leukocyte subsets. The mean fractions of CD19⁺ B cells, CD3⁺ T cells, and CD14⁻ monocytes prior to mitoxantrone treatment were 10.8%, 64.8%, and 24.4%, respectively, whereas the mean fraction after 6 cycles of mitoxantrone treatment were 4.5%, 65.4%, and 30.1%, respectively (P < .005). This suggests that B cells are more susceptible to the actions of mitoxantrone than T cells and monocytes. In B cell subsets, mitoxantrone preferentially affected CD27⁺ memory B cells; the mean fractions of CD27⁺ memory and CD27⁻ naive B cells before mitoxantrone treatment were 30.2% and 69.8%, respectively, whereas the mean fractions after 3 to 6 cycles of mitoxantrone treatment were 11.3% and 88.7%, respectively (P < .001; Figure 2).

ADVERSE EVENTS OBSERVED DURING TREATMENT AND FOLLOW-UP

The most common adverse effect following mitoxantrone treatment was transient nausea lasting 3 to 5 days. Other adverse events included a mild and transient elevation of serum glutamic pyruvic transaminase/serum glutamic oxaloacetic transaminase levels (4 patients), mild hair loss (5 patients), and transient amenorrhea (4 patients). Leukopenia was found in all patients to various degrees but resolved prior to the next mitoxantrone infusion. Two patients showed persisting neutropenia, with degrees but resolved prior to the next mitoxantrone infusion. The most common adverse effect following mitoxantrone treatment was transient nausea lasting 3 to 5 days. Other adverse events included a mild and transient elevation of serum glutamic pyruvic transaminase/serum glutamic oxaloacetic transaminase levels (4 patients), mild hair loss (5 patients), and transient amenorrhea (4 patients). Leukopenia was found in all patients to various degrees but resolved prior to the next mitoxantrone infusion. Two patients showed persisting neutropenia, which caused a delay in mitoxantrone administration. However, it was not related to infection and recovered without granulocyte–colony stimulating factor therapy. Although minor infections, such as urinary tract and upper respiratory tract infections, occurred in 4 patients, they were not related to mitoxantrone treatment.

<table>
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<tr>
<th>Patient No./Sex/Age</th>
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<td>Attacks in y Before, No. ARR After EDSS at Last Treatment</td>
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</table>

Abbreviations: Ab, antibody; ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; MTX, mitoxantrone; NMO, neuromyelitis optica; NMOSD, NMO spectrum disorder; PD, prednisolone.

a Immediate prebaseline therapy prior to mitoxantrone treatment; 1 indicates 12 mg/m² intravenous infusions for the 3-month induction followed by 6 to 12 mg/m² every 3 months; 2, 12 mg/m² infusions for 6-month induction followed by 6 to 12 mg/m² every 3 months.
Figure 1. Relapses in patients with neuromyelitis optica before and after treatment with mitoxantrone. When the frequency of relapses is compared between 1 year preceding the start of mitoxantrone therapy and 1 year after mitoxantrone therapy, a significant decrease in relapses is observed. On the x-axis, 0 indicates the start date of treatment. Each interrupted line on the y-axis represents a patient.

Figure 2. Effect of mitoxantrone on subgroups of B cells. Surface expression of CD19 and CD27 measured by flow cytometry for 4 representative patients with neuromyelitis optica shows preferential decrease in the fraction of CD27⁺CD19⁺ memory B cells (upper right panel) after treatment with mitoxantrone.
respiratory tract infections, were notable, no patients experienced a severe infection. All patients kept a left ventricular ejection fraction (LVEF) within the reference range on echocardiogram during the mean 17 months of mitoxantrone treatment. However, 1 patient who showed an asymptomatic reduction of LVEF (54%) on echocardiogram compared with a baseline LVEF of 64% discontinued therapy after a cumulative dose of 72 mg/m² of mitoxantrone. Neither mitoxantrone-related acute leukemia (therapy-related acute leukemia) nor congestive heart failure were found during the mean follow-up 41 months after completing mitoxantrone therapy until May 2010.

In this retrospective study, mitoxantrone treatment was shown to have a positive effect on the clinical course of patients with NMO who were highly relapsing and largely refractory to other immunotherapies. The relapse rate was significantly reduced by 75%, and disability in all patients either stabilized or improved. Most relapses during mitoxantrone treatment occurred during the early stages of treatment, and these were considered to be due to insufficient immunosuppression. With successful immunosuppression following 6 cycles of monthly mitoxantrone infusion, however, only 1 relapse occurred. Even more intriguing is that disabilities improved in 90% of patients, and 7 of 11 patients who already had severe neurological impairments (EDSS score >6) at baseline could walk without aid or rest. These findings suggest that mitoxantrone may have clinical benefit even in patients with advanced NMO.

Without a placebo control group, it is impossible to determine whether this clinical benefit was unequivocally in response to the mitoxantrone treatment. In MS, the advanced neurological disabilities are difficult to restore, as the severe disability is primarily a feature of secondary progression. In NMO, by contrast, repeated attacks are the main cause of cumulative disability. Furthermore, patients with highly relapsing NMO may have no time to recover from neurological symptoms due to a series of relapses. Accordingly, simply preventing further relapses might restore the neurological disability to a certain degree. This assumption provides the grounds for an aggressive treatment plan in patients with highly relapsing NMO, although they already have severe neurological disability.

Mitoxantrone is a synthetic anthracenedione that intercalates with DNA, causing cross-linking and strand breaks. Mitoxantrone also inhibits topoisomerase II, interfering with DNA repair.15-17 This agent produces generalized immunosuppression by inhibiting the migration of monocytes and lymphocytes, inducing apoptosis in dendritic cells, decreasing secretion of proinflammatory cytokines such as tumor necrosis factor, interleukin 2, and interferon γ, inhibiting B cell function and macrophage-mediated myelin degradation, and enhancing T-cell suppressor function.18-21 Interestingly, recent articles have indicated that mitoxantrone promotes differential inhibitory effects on the subgroups of leukocytes.
also a concern. A recent meta-analysis on therapy-related acute leukemia in mitoxantrone-treated patients with MS reported a cumulative incidence of approximately 0.8%.24 In a study by Ellis and Boggild,25 the median onset of therapy-related acute leukemia following mitoxantrone treatment was 18.5 months (range, 4-60 months), and more than 80% of cases occurred in patients exposed to more than 60 mg/m² of mitoxantrone. As our patients were treated with mitoxantrone before this serious safety concern was raised, the average cumulative dose was 100 mg/m². However, none of the patients had serious adverse effects during treatment or after its completion during the mean follow-up period of 41 months.

This study had some limitations. In particular, owing to the uncontrolled and retrospective nature of case series and based on clinical experience with few referral centers, it is difficult to provide a definitive evaluation of the clinical benefit of mitoxantrone. In addition, the possibility that the marked decrease in relapse rate is related to regression to the mean cannot be completely excluded.20

Finally, as mitoxantrone therapy was initiated a relatively short time after last relapse, the change in EDSS scores might be confounded by attacks that occurred near the beginning of mitoxantrone therapy, although most patients had already reached the baseline EDSS before the initiation of mitoxantrone. Nevertheless, considering the lack of controlled clinical trials and the difficulties inherent in conducting prospective randomized controlled trials in a rare disease, such robust effects as those shown in our case series provide some rationale for the use of mitoxantrone, particularly in the subgroup of patients who are highly relapsing despite immunotherapies.

Some physicians hesitate to use mitoxantrone owing to its serious adverse effects. However, the potential benefit of relapse prevention appears to outweigh the risks of adverse effects, especially in patients with aggressive relapsing NMO. In patients with MS who show severe disease activity, induction therapy with a powerful immunosuppressant such as mitoxantrone followed by maintenance therapy with immunomodulating agents appears to be beneficial.27-30 Considering the recent safety issue and the current findings in our case series that relapse rarely occurred after successful completion of 6 cycles of mitoxantrone induction, similar treatment strategies can be applied in NMO, such as monthly administration of up to 72 mg/m² of mitoxantrone for 6 months as induction therapy followed by safer immunosuppressive agents as maintenance therapy.

The question will be, how long can the benefits of short-term aggressive treatment with mitoxantrone continue and what kind of drug and treatment schedule are more beneficial? These issues must be addressed in the future.

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