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REVIEW

Role of Immunosuppressive Therapy for the Treatment of Multiple Sclerosis

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Abstract Immunosuppressives have been used in multiple sclerosis (MS) since 1966. Today, we have many treatments for the relapsing forms of the disease, including 8 US Food and Drug Administration-approved therapies, with more soon to be introduced. Given the current treatment landscape what place do immunosuppressants have in combating MS? Trial work and our experience suggest that immunosuppressives still have an important role in treating MS. Cyclophosphamide finds use in treating patients with severe, inflammatory relapsing remitting MS or those suffering from a fulminant attack. We tend to employ mycophenolate mofetil as an add-on to injectable therapy for patients experiencing breakthrough activity. Some progressive (primary progressive multiple sclerosis or secondary progressive multiple sclerosis) patients may stabilize after treatment with either cyclophosphamide or mycophenolate. We rarely employ mitoxantrone because of potential cardiac or carcinogenic effects. We prefer to use cyclophosphamide or mycophenolate mofetil in preference to methotrexate because evidence of efficacy is limited for this drug. We have less experience with azathioprine, but it may be an alternative for patients with limited options who are unable to tolerate conventional therapies.

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Introduction

Immunosuppressives have been employed successfully in patients with autoimmune disease. Patients with diseases as diverse as Wegener's granulomatosis, myasthenia gravis, systemic lupus erythematosis, and rheumatoid arthritis benefit from immunosuppressives. Consequently, it is logical that trials of immunosuppressives would be given to multiple sclerosis (MS) patients, given that MS is an autoimmune disease, and cyclophosphamide was found to suppress an animal model of MS, allergic autoimmune encephalitis [1]. Aimard et al. [2] reported the first use of an immunosuppressant in MS. This proof of concept experiment opened the door to more widespread use of immunosuppressives.

Today, the landscape of treatment has changed so that we have many effective and generally well-tolerated and safe Food and Drugs Administration (FDA)-approved drugs for relapsing forms of MS in our armamentarium. Immunosuppressive medications are off-patent and, as a result, have no funding for large-scale trials to more definitively characterize their appropriate use in MS patients. They are currently not FDA-approved for MS and thus are used off-label. Nonetheless, immunosuppressives continue to be used in MS patients—appropriately so, given that some patients are refractory to more conventional therapies or find themselves in a particular situation that requires immunosuppression (e.g., tumefactive MS) In this review article we will discuss the use of cyclophosphamide, mitoxantrone, mycophenolate mofetil, azathioprine, and methotrexate in MS. We will recap the mechanism of action of each of these

drugs, effects on the immune system relevant to MS, trial results, and side effect profiles.

Cyclophosphamide

Mechanism of Action

Cyclophosphamide is a nitrogen mustard prodrug that undergoes hepatic conversion to form active metabolites. These compounds cause cell death by leading to inter- and intrastrand DNA crosslinking. This occurs because phosphoramide mustard adds an alkyl group to the guanine bases found in the DNA. More rapidly proliferating cells are more susceptible to cyclophosphamide. Consequently, the bone marrow, bladder, and gastrointestinal epithelium are disproportionately affected.

Cyclophosphamide depletes lymphocytes in both the peripheral blood and cerebrospinal fluid, and prompts an associated decrease in immunoglobulin production [3, 4]. It is equally distributed through the blood and cerebrospinal fluid [5]. It reduces both B and T lymphocytes, though preferentially affects CD4+ T cells. Immunologic work suggests that cyclophosphamide may drive the immune system away from a T helper type 1 (Th1) immune stance that can be deleterious in MS towards a more favorable T helper type 2 (Th2) profile [6–8]. Cytokines typically associated with a Th2 response are increased in cyclophosphamide-treated patients. An increased Th2 response to myelin auto-antigens has been observed [8]. Hafler et al. [6] reported that patients who shifted to a Th2 phenotype after cyclophosphamide treatment showed benefit from the drug. Similarly, Comabella et al. [9] found that patients with normalized interleukin-12 (a cytokine found in Th1 responses) after treatment with cyclophosphamide had less active MS. Cyclophosphamide has also been shown to encourage a Th2 phenotype and reverse increased interferon (IFN)-gamma production of CD8+ T cells in patients with secondary progressive MS [10].

Clinical Effects and Use

Cyclophosphamide is indicated for use in the treatment of malignancies and nephritic syndrome. It is used off-label for the treatment of many diseases, including lupus nephritis and MS. Initial open label trials demonstrated a beneficial effect in both relapsing and secondary progressive patients [11, 12]. A randomized trial conducted by Hauser et al. [13] found that progressive patients receiving cyclophosphamide in combination with adrenocorticotropic hormone (ACTH) did better than patients receiving plasmapheresis in combination with low-dose oral cyclophosphamide and ACTH or ACTH alone. Based on these results two placebo-controlled trials were conducted in progressive populations with different dosing regimens: the Canadian cooperative trial [14] and the Kaiser study [15]. Neither trial demonstrated a benefit to cyclophosphamide when compared with placebo in the group studied. Three factors emerge to explain the discrepant results of the Boston trials versus the Canadian cooperative trial and Kaiser study. First, the Boston group enrolled patients who were younger and had a shorter disease duration. Second, patients in the Boston trial had progressive disease less often (40 % in the Boston trial *vs* 60 % in the Canadian trial.) Third, the dosing regimen was different in each trial. In our view it is likely that the Canadian cooperative trial and Kaiser study were correct in demonstrating a lack of effect in a progressive, noninflammatory older populations given lower doses of cyclophosphamide less frequently. Other drugs, such as beta-IFN and rituximab, have also been found to be less effective in noninflammatory later progressive stages. Further support for this interpretation comes from subsequent open-label studies, which consistently demonstrate a benefit in younger, inflammatory, less progressive populations [16–19]. Though initial trials only studied clinical outcomes, subsequent trials demonstrate that cyclophosphamide also has a robust effect on magnetic resonance imaging (MRI). Smith et al. [20] reported an 82 % reduction in gadolinium enhancement compared with the pretreatment group. Studies generally show that the onset of MRI effect is within 1–5 months [21]. Cyclophosphamide has also been used successfully in combination with IFN [22–24]. Despite different dosing schedules trials with cyclophosphamide have been consistent in showing that patients respond best if they are younger, have a shorter disease duration, and still have relapses or evidence of recent MRI activity [25, 26]. In fact, cyclophosphamide has also been employed successfully in pediatric MS [27, 28]. A retrospective review conducted at our center showed that patients with primary progressive disease generally do not respond well to cyclophosphamide treatment [29].

Zipoli et al. [30] conducted a retrospective comparison of 78 cyclophosphamide-treated patients and 75 mitoxantrone-treated patients with either relapsing or progressive disease. Mitoxantrone was administered at a dosage of 8 mg/m^2 monthly for 3 months, then every 3 months, until a dosage of 120 mg/m^2 was reached. Cyclophosphamide was administered at a dosage of 700 mg/m^2 monthly for 12 months, then bimonthly for another 24 months. Time to first relapse (2.5 years in cyclophosphamide *vs* 2.6 in mitoxantrone, p= 0.5) and MRI effect (63 % gadolinium reduction cytoxan *vs* 69 % with mitoxantrone, p=0.1) did not differ between groups. Time to disease progression was slightly longer in the mitoxantrone group (3.6 years *vs* 3.8, p=0.05.)

Different dosing regimens have been tested. Doses escalated to 2000 mg/m^3 are not well tolerated [31]. At our center we

target a white blood cell count nadir of 1500–2000/mm^3. We start at a dose of 800 mg/m^3 and escalate to a maximum dose of 1600 mg/m^3. We find that infectious complications are uncommon. White blood cell nadir depends on dosing strategy, but with a monthly pulse, usually occurs 8–14 days after infusion. Patients who only receive induction doses have disease recurrence as early 6 months after administration and usually by 2 years [32]. This observation led to the development of redosing strategies. At our center we dose monthly for the first year, every 6 weeks the second year, and every 8 weeks in the third year. Further details of administration recommendations can be found in Table 1. Steroids are generally given with cyclophosphamide, but efficacy has been seen in both steroid-treated patients and those not receiving steroids.

Induction

Because cyclophosphamide crosses the blood–brain barrier [4] and thus has access to the central nervous system [5] there is a rationale for its use in patients with severe steroid refractory exacerbations. We have had success with this approach in a number of patients (see inset case 1 and case 2). Although approaches differ we have most typically given an in-hospital induction regimen with 3–5 days of recurrent therapy. A report by Krishnan et al. [33] describes an induction regimen of 50 mg/kg/day of cyclophosphamide for 4 days with granulocyte colony-stimulating factor to rescue neutrophils. Enrolled patients were required to have consecutive active MRIs, at least 1 clinical exacerbation in the year prior to high-dose cyclophosphamide treatment, or a sustained increase of 1 point or higher on the Expanded Disability Status Scale (EDSS) in the preceding year. After a mean 23 months of follow-up when compared to clinical metrics prior to study entry, EDSS improved, on average, 2.11 points (p=0.02) and a reduction of 81 % (p=0.01) in gadolinium-enhancing lesions was achieved. A follow-up study by the same group [34] evaluated 40 patients who had been treated with the cyclophosphamide-conditioning regimen described earlier and then treated with glatiramer acetate. A pre/-post-treatment analysis revealed that there was a reduction in annualized relapse rate from 1.37 to 0.27 during the study period. There was also a reduction from 0.86 mean gadolinium-enhancing lesions at baseline to no lesions from 0 to 12 months and 0.08 at 15–24 months. Fifty-five percent of patients had no evidence of disease activity at a mean follow-up of 14 months. We have observed that induction protocols can have a rapid effect (days) on MRI activity.

Cyclophosphamide Induction Case #1

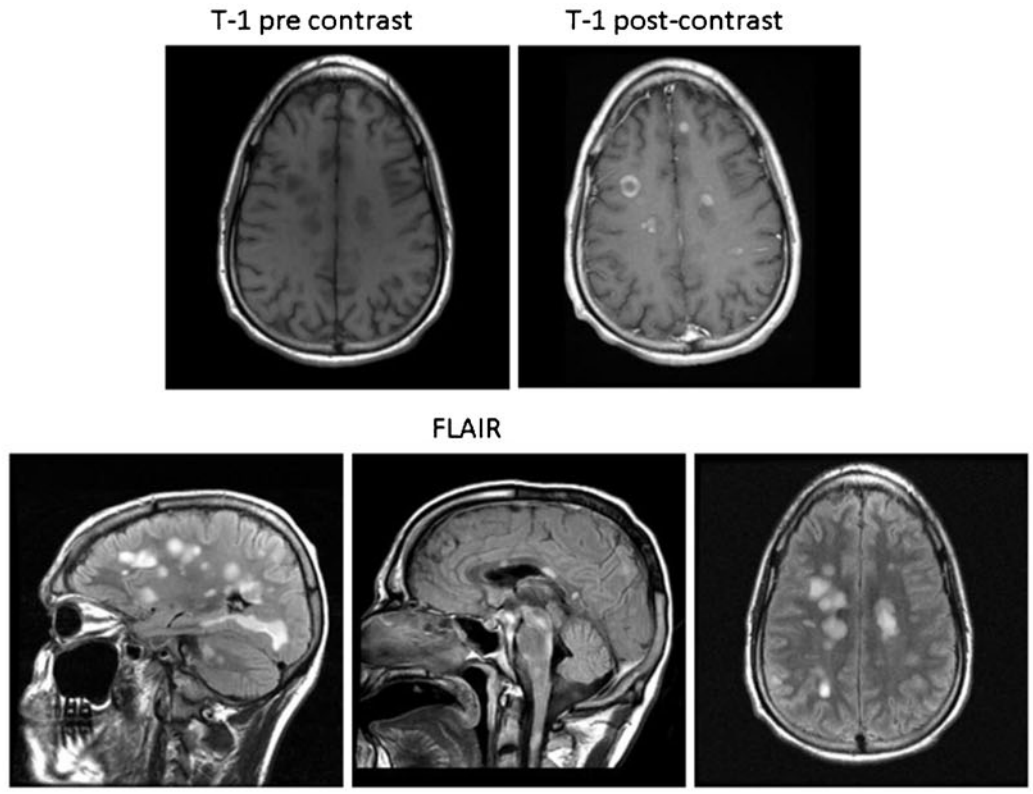
A 26-year-old man with no significant past medical history presented initially with blurry vision in the left eye, which progressed to become bilateral. MRI was obtained (Fig. 1; MRI 7 December 2011). He received a course of methylprednisolone. Despite this, his symptoms worsened and he developed left-sided numbness and weakness with a continued progressive decline in his vision. He then developed severe ataxia and was no longer able to ambulate. He was treated with another course of methylprednisolone and underwent 4 plasma exchange procedures. A repeat MRI was performed, which showed continued activity (Fig. 1; 20 December 2011). He continued to deteriorate, exhibiting diffuse weakness, encephalopathy, and dysphagia. He was intubated after experiencing difficulty in breathing. A cyclophosphamide induction protocol was initiated followed by another course of plasmapheresis. Recovery began, but was slow, and so he was given another cyclophosphamide induction. After this second course, he showed improvement. His encephalopathy lifted and he was successfully extubated. He was discharged to a rehabilitation facility. When initially seen in follow-up at our clinic he could walk long distances with a cane, though he remained ataxic. He had persistent left body numbness and complaints of cognitive dysfunction. MRI was obtained (Fig. 1; 16 January 2012). At follow-up 6 months later he had improved to the

Table 1 Cyclophosphamide protocol

White blood cell (WBC) nadir*	Dose adjustment
>2000 mm^3	Increase dosage in increments of 200 mg/m^2 until a dose of 1400 mg/m^2, then by 100 mg/m^2
1500–2000 mm^3	Maintain current dose
<1500 mm^3	Decrease dosage 100–200 mg/m^2 depending on history of WBC counts, current nadir
Start at 800 mg/m^2†; maximum monthly dose is 1600 mg/m^2. Maximum suggested cumulative dose is 80 g Frequency of administration: monthly for year 1, every 6 weeks for year 2, every 8 weeks for year 3, then at the physician's discretion	

*Drawn day 8, 11, and 14 after infusion

†Round dose to nearest 100 mg


T-1 pre contrast
T-1 post-contrast
FLAIR
12/07/11
T-1 pre contrast
T-1 post-
FLAIR
1/16/12

◀ **Fig. 1** Effects of cyclophophamide induction in a 28-year-old patient with a fulminant multiple sclerosis attack. Figures taken in December 2011 are pre-cyclophosphamide induction; the image taken on 16 January 2012 is post-cyclophosphamide induction

extent that he was able to walk unassisted, had no detectable weakness, numbness, or ataxia, though he had some mild cognitive complaints.

Cyclophosphamide Induction Case #2

A 29-year-old female medical student with past medical history including Gilbert's disease and polycystic ovary syndrome developed behavioral changes 3 weeks prior to the admission. These changes included quarrels with friends, increased appetite, and hypersexual behavior. These symptoms were followed by fatigue, irritability, and depression. Three days prior to admission she became confused, with repetitive, sometimes nonsensical, speech. She came to medical attention when she failed to find her way home from work. There was no history of recent infection or vaccination.

On admission her mental status testing demonstrated disturbance in reality testing, abnormal judgment, and poor orientation to time, place and situation. She was anxious, irritable, and prone to paranoia. As the patient was not cooperative, further testing was limited. No cranial nerve abnormalities or motor defects were noted. Deep tendon reflexes were normal and plantar responses were bilaterally flexor.

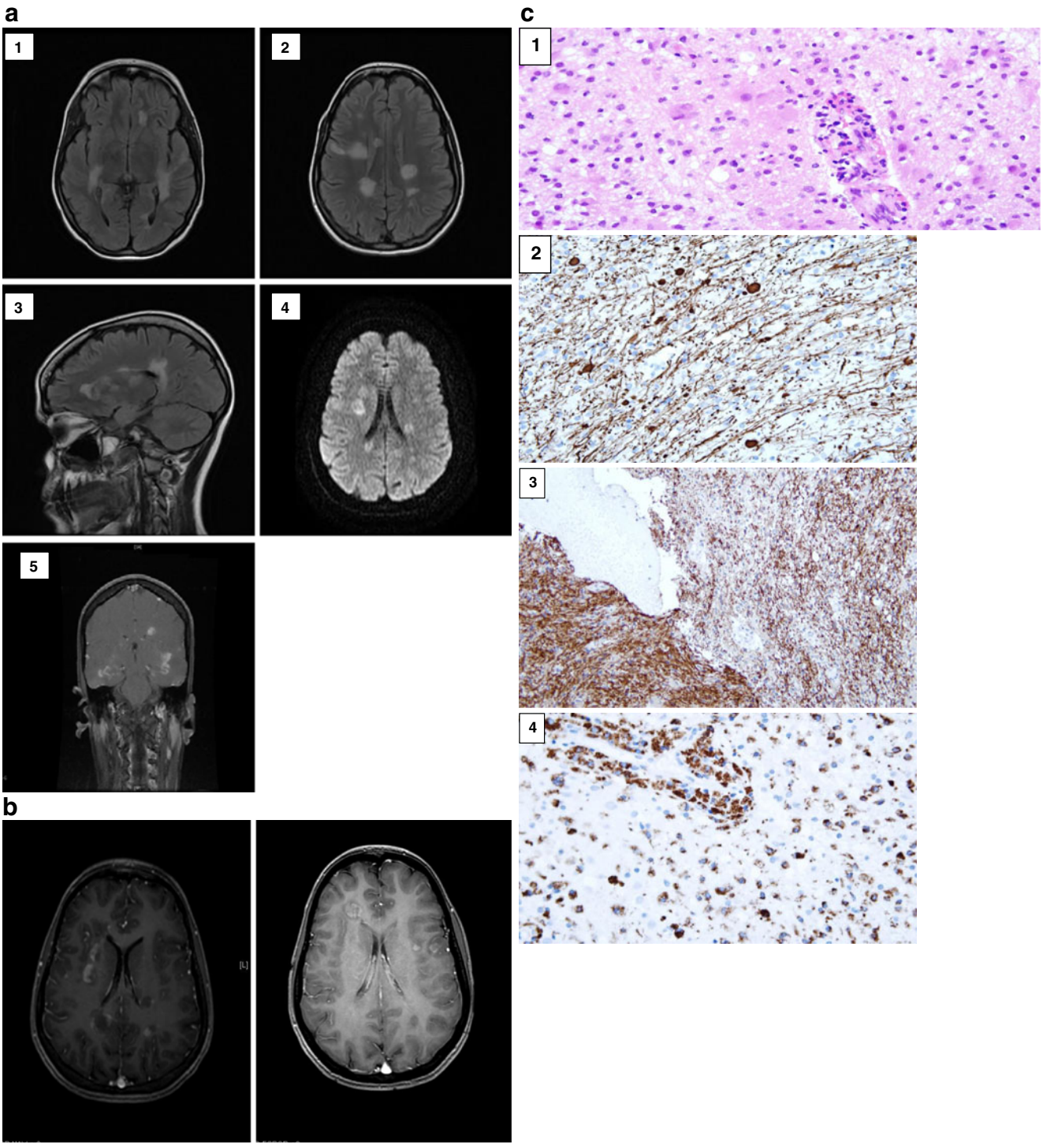
Routine blood examination and cerebrospinal fluid analysis were normal. Brain MRI scan showed bilateral supratentorial white matter lesions in the T2 and fluid attenuated inversion recovery (FLAIR) sequences, involving the centrum semi ovale, corpus callosum, internal capsule, and mesial -temporal lobes, including T1 postgadolinium enhancement. There was also evidence of restricted diffusion on the diffusion-weighted image sequence (Fig. 2a).

A working diagnosis of acute inflammatory demyelinating disease was made and treatment with steroids was initiated. She received 5 days of 1 g methylprednisolone intravenously daily, followed by another 5 days of 500 mg of methylprednisolone intravenously. After the 10-day course of high-dose steroid a prednisone taper was started, though no improvement was observed. She was then treated with immunoglobulin intravenously starting on day 10 (0.4 g/ kg/day for a daily dose of 20 g) for 5 consecutive days, but still there was no clinical improvement. Three weeks after her hospitalization, a brain MRI showed new enhancing lesions (Fig. 2b). A stereotactic biopsy of the right frontal lobe lesion showed an acute demyelinating lesion with extensive axonal damage (Fig. 2c). Induction immunosuppressive treatment with cyclophosphamide was given. Ten days after finishing the intravenous immunoglobulin treatment she received cyclophosphamide intravenously 600 mg/m^2/day and methylprednisolone. Eggs were harvested from her ovaries because of potential infertility. After treatment, gradual recovery occurred. The patient's judgment and insight improved. A cognitive assessment using the Montreal cognitive assessment test was performed and a score of 15/30 was obtained with deficit of the executive function, spatial perception, attention, abstract thinking, and orientation to time. She was discharged to a rehabilitation hospital where she continued cognitive rehabilitation. Follow-up brain MRI 1 month after discharge showed a hyperintense T2 lesion involving the white matter in both hemispheres. Most lesions were smaller than shown in the previous MRI scans. No new lesions were demonstrated and no gadolinium enhancement was detected (Fig. 2D). Whole spinal MRI was normal. One month after discharge a follow-up Montreal cognitive assessment test showed impressive improvement, with a score of 26/30. She was able to return to her clinical rotations. A follow-up brain MRI 3 months after admission showed further improvement with a significant reduction of the lesions load and no evidence of new disease activity (Fig. 2e).

Side Effects

Reported side effects include hemorrhagic cystitis, infection, bladder cancer, infertility, alopecia, nausea, and vomiting. In rheumatologic populations sustained amenorrhea has been reported in up to 57 % of women who received cyclophosphamide for 2 years [35]. In population of MS patients, 33 % of women developed sustained amenorrhea, with 17 % becoming menopausal [36]. Women receiving cyclophosphamide for rheumatologic causes were less likely to become infertile if younger (age<30 years) or receiving low cumulative doses (<300 mg/kg) [37, 38]. Portaccio et al. [36] reported amenorrhea occurring as early as the first dose or at a cumulative dose as low as 1500 mg. Given this, we believe in a fertile woman considering the use of cyclophosphamide it is essential to discuss oocyte cryopreservation. Oral contraceptives can be considered in an effort to decrease the risk of infertility, though evidence for this approach is weak [38]. A small study suggests that men may also develop decreased sperm counts and motility when taking cyclophosphamide [39].

A retrospective review of pulse cyclophosphamide found that infection occurred in 28 % of patients, with 9 % requiring hospitalization [40]. It is worth noting that the doses given to these patients are, on average, less than what is given to MS patients. Conversely, this patient group could have been immunosuppressed as a result of their underlying rheumatologic disease (e.g., system lupus erythematosus). Opportunistic infections and progressive multifocal



leukoencephalopathy (PML) have been reported with cyclophosphamide in other diseases, although patients may have been more susceptible to these complications than MS patients because they were either immune-compromised as a result of their disease or they were being given other immunosuppressive medications concomitantly [41, 42]. PML has not been reported in MS patients receiving cyclophosphamide. However, it is clear that patients treated with natalizumab are at increased risk if they have a history of prior immunosuppressant treatment, including cyclophosphamide [43]. An increased incidence of malignancy has been reported both in patients with cancer treated with cyclophosphamide and with rheumatic diseases. Risk appears to increase once patients have received an accumulated dose >

◀ **Fig. 2** (**a**) Cyclophophamide case 2; brain magnetic resonance (MR) images on admission. 1 = fluid attenuated inversion recovery (FLAIR) axial section at the centrum semi-ovale level shows bilateral parietal and frontal white matter hyperintense lesions without mass effect. 2 = FLAIR axial section at the basal ganglia and thalamus level shows bi-medial temporal and frontal white matter hyperintense lesions. 3 = FLAIR sagittal section shows periventricular and corpus callosal hyperintense lesions. 4 = diffusion-weighted image of axial section, lesion shows restricted diffusion. 5 = Coronal section through the hippocampus shows right frontal and bi-temporal gadolinium-enhancing lesions and bilateral hippocampal swelling. (**b**) MR images 3 weeks after admission. Axial section shows new right frontal lesion that underwent enhancement of gadolinium. (**c**) Brain biopsy. 1 = hematoxylin and eosin stain shows inflammatory infiltrate around blood vessels, and in the tissue and reactive astrocytes. 2 = neurofilament (NF) stain shows axonal spheroid bodies. 3 = myelin basic protein (MBP) stain demonstrates the loss of myelin. 4 = CD 68 positivity shows macrophages infiltrate around blood vessels. (**d**) MR images 1 month after discharge. 1=FLAIR sagittal section shows decreased FLAIR lesion burden when compared with prior MRI scan. 2=FLAIR axial section shows bi-hemispheric hyperintense lesions with decreased lesion load. 3 = Fast spoiled-gradient echo (FSPGR) with gadolinium, axial section—no enhancing lesions were found. (**e**) Brain MRI 3 months after discharge. Axial T2 FLAIR shows further reduction of the hyperintense lesions

80 g [44–46]. Talar-Williams et al. [45] reported that 5 % of patients receiving cyclophosphamide for treatment of Wegener's disease developed bladder cancer. Six of 7 of these cases occurred in patients treated over 2 years and receiving a cumulative dose >100 g. Given this and other reports [47], we believe it is prudent to perform a yearly urine cytology and cytoscopy after 3 years.

With aggressive fluid hydration of 2 L the day of infusion and 3 L the day after we have found hemorrhagic cystitis exceedingly rare. For this reason we do not co-administer mesna with our infusions. Experience in the treatment of lupus nephritis with pulse cyclophosphamide and methylprednisolone indicates that concomitant administration of methylprednisolone may help decrease renal toxicity and even augment cyclophosphamide's efficacy [48]. Therefore, typically, we co-administer the drugs. Liberal use of anti-emetics can help reduce nausea and vomiting.

Despite these concerns, a recent follow-up study of MS patients undergoing pulse cyclophosphamide treatment found that it was well tolerated, with amenorrhea and hemorrhagic cystitis being the most common side effects of note. Alopecia developed in a third of patients and hemorrhagic cystitis in 4.5 %. Malignancies were seen in 4 patients (3.6 %), 3 of whom were treated previously with azathioprine. Interestingly, > 80 % of patients surveyed after treatment judged cyclophosphamide as either very or relatively acceptable, and tolerable [36]. In our experience cyclophosphamide has been proven as a safe drug, the side effects of which can be managed.

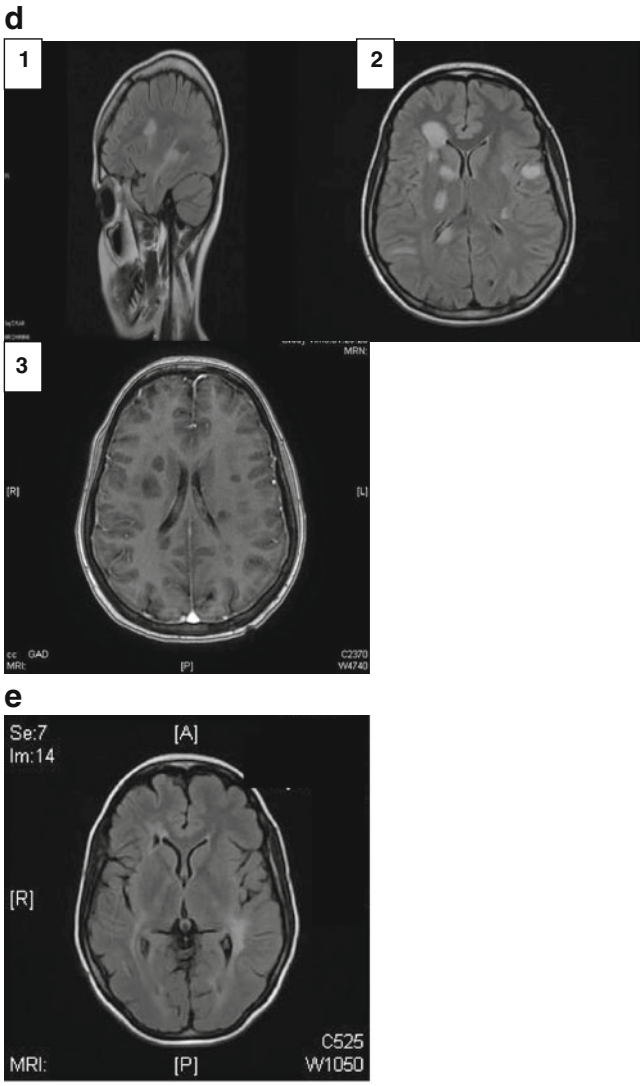


Fig. 2 (continued)

Mycophenolate Mofetil

Mechanism of Action

Mycophenolate mofetil (Cellcept) is a potent immunosuppressant that selectively inhibits an enzyme responsible for the *de novo* synthesis of the DNA nucleotide guanine within T-cells, B-cells, and macrophages. It is used commonly post-transplant to prevent graft versus host disease.

Clinical Effects and Use

Though no blinded placebo controlled trials have been completed in MS, open-label trial reports are available. Ahrens et al. [49] gave mycophenolate to 7 relapsing or progressive patients, followed them for 1–18 months and found 5 patients that remained static or improved. Frohman et al. [50] conducted a retrospective review of 79 patients treated with

mycophenolate either alone or in combination. Predominantly secondary progressive MS patients were included and followed, on average, for 1 year. Seven patients had disease progression, while others were perceived as remaining stable in time. An open-label study in 30 active relapsing-remitting (RR) patients treated with a combination of IFN beta-1a and mycophenolate reported a reduction in relapse rate (2 to 0.57), improvement in mean EDSS (2.9 to 2.6), and absence of gadolinium enhancing lesions on MRI follow-up [51]. During the six month study, more relapses occurred in the first two months, suggesting some delay in drug effect. Frohman et al. [52] randomized 35 active relapsing-remitting patients to either mycophenolate mofetil or IFN-beta-1a intramuscular for 6 months. The study was open label for clinical assessments, but MRI readers were blinded. They reported that there was no difference between groups for new T2 or gadolinium lesions, suggesting an equivalence between these treatments. Clinical assessments were also performed, but, because the study was small and open-label, no definite conclusions can be made.

At our center, we tend to employ mycophenolate in selected cases of patients with either relapsing or progressive disease that, despite treatment with a first-line disease-modifying agent (IFN-beta or glatiramer acetate), continue to worsen. We have also had successful results in patients with neuromyelitis optica, and a retrospective study supports its use in this disease [53].

We use the same dosing used in the open-label studies: 250 mg twice daily for 1 week, 500 mg twice daily for week 2, 750 mg twice daily for week 3, and then 1 g twice daily thereafter. Laboratory studies are performed weekly during the first month, twice monthly during the second and third month then monthly and consist of liver function testing, a complete blood count, and basic metabolic panel.

Side Effects

Common side effects associated with mycophenolate use include diarrhea, nausea, abdominal pain, insomnia, dizziness, increased susceptibility to infections, and leukopenia. There is also an increased risk of lymphoma. Mycophenolate is contraindicated in pregnancy. The recent study by Frohman et al. [52] showed that diarrhea and headache were seen in almost a third of patients, though no mycophenolate discontinuations occurred as a result. Abdominal pain and pruritis were also seen more frequently than placebo. Serious infections did not occur. Other studies have been consistent with this safety and tolerability profile [49–51]. Opportunistic infections, including PML have occurred in mycophenolate-treated solid organ transplant and lupus patients. PML has not been reported in a mycophenolate-treated MS patients. Patients developing PML were all on concomitant immunosuppressive medications and may also have had some disease-related immunosuppression [54].

Azathioprine

Mechanism of Action

Azathioprine (Imuran) is a purine analogue that is metabolized to 6-mercaptopurine and thioinosine acid, which compete with DNA nucleotides, causing immunosuppression [55]. It has found use in autoimmune disorders, such as myasthenia gravis, and to prevent post-transplant organ rejection.

Clinical Effects and Use

We use cyclophophamide or mycophenolate mofetil in preference to azathioprine due to conflicting results about its efficacy in MS. A meta-analysis concluded that azathioprine probably does attenuate relapsing remitting MS [56]. This study reviewed the effects of this drug in nearly 700 patients enrolled in placebo-controlled double-blind randomized trials. When pooled, these trials showed a relative risk reduction of relapse of 23 % (95 % confidence interval 12–33 %) compared with placebo. Only 87 patients could be included in an analysis of whether azathioprine reduced risk of disability progression. At 3 years the risk of progression was reduced by 42 % and was statistically significant. More recent trials have reported conflicting results. Havrdova et al. [57] assessed whether patients taking intramuscular IFN-beta-1a would benefit from the addition of azathioprine. They randomized 181 patients to three groups: IFN alone, IFN with 50 mg/day azathioprine or IFN with 50 mg/day azathioprine, or IFN with 50 mg/day azathioprine and prednisone 10 mg orally every other day. No difference in annualized relapse rate or cumulative probability of sustained disability was seen at 2 years. MRI analysis showed no change in percentage of brain volume loss between treatments, and T2 lesion volume measures were also, generally, nonsignificant. These results remained similar when follow-up was performed after 6 years of treatment [58]. Less than 1 % of patients were disease-free at 6 years. Etemadifar et al. [59] conducted a study comparing azathioprine head-to-head with IFN treatment. Ninety-four patients with early relapsing remitting MS were randomized to either treatment with an IFN-beta medication or 3 mg/kg/day of azathioprine. After 1 year the proportion of patients relapse-free was greater in the azathioprine group (77 %) than the IFN group (57 %) ($p<0.05$). Mean EDSS was also improved (0.96 in azathioprine group *vs* 1.34 in IFN-beta, $p<0.01$.) Patients were aware of their treatment allocation, though the examining neurologists were blinded.

Side Effects

Leukopenia, macrocytic anemia, and liver function abnormalities can be seen with azathioprine treatment. Leukopenia typically becomes less frequent with time. For example, in

the British and Dutch trial [60] 26 % of patients were leukopenic at the end of year 1, while only 8 % were leukopenic at the end of year 3. There is debate about whether long-term azathioprine use predisposes to cancer [61–63]. Lhermitte et al. [61] followed 131 patients over about 10 years and identified 10 cancers, while age- and sex-matched controls developed only 4 cancers. On average, patients were treated with 100 mg/day for 6 years. Reported cancers were solid tumors rather than hematologic. A case control study undertaken using the Lyon MS database [62] found that 14 patients from a database of 1191 patients developed cancer. Risk of developing cancer with over 5 years of azathioprine treatment was double that in patients not treated with azathioprine, and more than 4-fold higher when treated for more than 10 years. When examining cumulative dose rather than duration of treatment, similar effects were found, with 5 years of treatment being equivalent to a cumulative dose of 300 g. Solid tumors were more common than hematologic malignancies. In contrast to the prior 2 studies, Amato et al. [63] found that azathioprine-treated MS patients were less likely to develop cancer when compared with untreated patients.

Mitoxantrone

Mechanism of Action

Mitoxantrone intercalates DNA functioning to both suppress and modulate the immune system. It is the only medication approved by the FDA for the treatment of secondary progressive multiple sclerosis (SPMS), although it also carries an indication for RRMS and progressive relapsing MS.

Trial Effects and Use

Novantrone is approved for MS, treatment of hormone-refractotory prostate cancer, and acute nonlymphocytic leukemias. In MS it is indicated for use in secondary progressive, progressive relapsing, or worsening relapsing remitting MS, but not primary progressive disease. Two randomized controlled trials suggest that mitoxantrone is efficacious in these settings [64, 65]. Both trials demonstrated a good effect on relapses. A *post hoc* analysis of 1 of the 2 trials [65] found that patients with nonrelapsing secondary progressive MS responded to mitoxantrone, as well as secondary progressive patients with relapses, with favorable effects on EDSS progression and relapse rate seen in both groups.

Side Effects

Mitoxantrone has many potential toxicities, including cardiotoxicity, acute leukemias, leukopenia, depression, bone pain renal failure, nausea, vomiting, alopecia, predisposition to urinary tract infection, amenorrhea, and teratogenicity. In our view, the side effects of primary concern are development of acute leukemias and cardiac failure. Recent estimates are that acute leukemias occur in about 1 % of mitoxantrone treated patients and cardiac systolic dysfunction in about 10 % with heart failure in about 1 % [66]. At our center we rarely use mitoxantrone because of its side effect profile.

Methotrexate

Mechanism of Action

Methotrexate is a general immunosuppressant that acts primarily by inhibition of dihydrofolate reductase [55].

Trial Effects and Use

Methotrexate is indicated for use in cancer, psoriasis, and rheumatoid arthritis, and has been used off-label for the treatment of MS. We prefer other immunosuppressives, like cyclophosphamide or mycophenolate mofetil, because few successful treatment trials of methotrexate have been conducted in MS patients. Neumann et al. [67] saw no difference from placebo in an open-label trial of 15 patients receiving alternating 2.5 mg of methotrexate and 6 mg of mercaptopurine. Currier et al. [68] conducted a trial in 44 MS patients, both relapsing and progressive. At 18 months the mean number of relapses in patients with relapsing remitting MS was less in the methotrexate-treated group ($p=0.05$). No effect was seen on the progressive population. A more recent trial by Ashtari et al. [69] randomized 80 relapsing remitting MS patients to treatment with either intramuscular IFN-beta-1a or methotrexate 7.5 mg/week. After 1 year, both groups experienced a reduction in relapse rate compared with the year prior to beginning the trial (methotrexate 1.75→0.97, IFN 1.52→0.57, $p<0.01$), but a strong trend ($p=0.06$) favored the IFN group.

Methotrexate has been studied with IFN-beta as a potential combination therapy. In an open-label trial, Calabresi et al. [70] added 20 mg of methotrexate to intramuscular IFN-beta-1a for 6 months in 15 relapsing patients and found a 44 % reduction in number of gadolinium-enhancing lesions when compared with baseline scans of patients on IFN-beta-1a alone. Cohen et al. [71] randomized patients with breakthrough disease on intramuscular IFN-beta-1a to combination treatment with either oral methotrexate 20 mg weekly, methylprednisolone 1 g intravenously bimonthly, or both. Patients receiving combination therapy did not experience any additional benefit from the addition of methotrexate either with or without methylprednisolone. Endpoints included new or enlarged T2 lesions, gadolinium-enhancing lesions, MS functional composite, and brain parenchymal fraction.

A small trial suggested slight benefit for progressive MS patients treated with methotrexate when compared to placebo. Goodkin et al. [72] randomized 31 progressive patients to 7.5 mg methotrexate and 29 to placebo. Typical outcome measures, including time to first relapse, sustained EDSS progression, and change in T2 or gadolinium-enhancing lesions [73], did not differ between groups. The trial did, however, reveal that patients taking methotrexate benefitted on its primary outcome measure, a combination of EDSS, ambulation index, box and block test, and 9-hole peg test. An open-label study found that 89 % of unresponsive progressive patients receiving methotrexate intrathecally every 8–11 weeks for 8 cycles were improved compared with their baseline [74].

Side Effects

Methotrexate can cause nausea, vomiting, diarrhea, headache, dizziness, mouth sores, skin rashes, joint aches, and hair thinning. Potentially serious side effects include infertility, teratogenicity, lymphoma, bone marrow suppression, pneumonitis, renal failure, and gastrointestinal and hepatotoxicities. Despite these potential side effects, methotrexate appears relatively safe for use in MS patients. MS patients exposed to methotrexate tolerated the treatment as well as placebo and have experienced no serious adverse events. Because efficacy has not been clearly demonstrated in trials we rarely use methotrexate in our patients.

Conclusion

Though our understanding of how immunosuppressives affect MS and how well they work is somewhat limited, they continue to find use in selected patients. The two drugs we use in our practice are cyclophosphamide and mycophenolate. We use cyclophosphamide for severe inflammatory relapsing and acute (fulminant) MS and mycophenolate in selected non-responders with relapsing or progressive multiple sclerosis. Further work characterizing the immunologic effects of these drugs and which patients respond to treatment will be useful for clinicians. Such research would also help expand our overall understanding of the underlying pathophysiology of MS. Ultimately, the more we know about these particular drugs the better we will be able to inform and treat our MS patients.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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