

Appendix A: Extended Disability Status Scale (EDSS)

APPENDIX A

Kurtzke Expanded Disability Status Scale (EDSS)

Note 1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory, and the precise step number is defined by the Functional System (FS) score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation, and usual equivalents in Functional System scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS. Each step (e.g., 3.0 to 3.5) is still part of the DSS scale equivalent (i.e., 3). Progression from 3.0 to 3.5 should be equivalent to the DSS score of 3.

- 0 - Normal neurological exam (all grade 0 in FS).
- 1.0 - No disability, minimal signs in one FS (i.e., grade 1).
- 1.5 - No disability, minimal signs in more than one FS (more than on FS grade 1).
- 2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1).
- 2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).
- 3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
- 3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).
- 4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest 500 meters.
- 4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance: characterized by relatively severe disability usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.
- 5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions): (usual FS equivalents are one grade

5 alone, others 0 or 1: or combinations of lesser grades usually exceeding specifications for step 4.0).

- 5.5 - Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities: (usual FS equivalents are one grade 5 alone, others 0 or 1: or combination of lesser grades usually exceeding those for step 4.0).
- 6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting: (usual FS equivalents are combinations with more than two FS grade 3 +).
- 6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting (usual FS equivalents are combinations with more than two FS grade 3 +).
- 7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to a wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (usual FS equivalents are combinations with more than one FS grad 4 +; very rarely pyramidal grade 5 alone).
- 7.5 - Unable to take more than a few steps; restricted to wheelchair, may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair; (usual FS equivalents are combinations with more than one FS grade 4 +).
- 8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day, retains many self-care functions; generally has effective use of arms; (usual FS equivalents are combinations, generally grade 4 + in several systems).
- 8.5 - Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions; (usual FS equivalents are combinations generally 4 + in several systems).
- 9.0 - Helpless bed patient: can communicate and eat; (usual FS equivalents are combinations, mostly grade 4 +).
- 9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (usual FS equivalents are combinations, almost all grade 4 +).
- 10.0 - Death due to MS.

Appendix B: Ambulation Index (AI)

APPENDIX B

Ambulation Index (AI)

- 0 = Asymptomatic; fully active.
- 1 = Walks normally but reports fatigue which interferes with athletic or other demanding activities.
- 2 = Abnormal gait or episodic imbalance; gait disorder is noticeable to family and friends. Able to walk 8 meters in 10 seconds or less.
- 3 = Walks independently; able to walk 8 meters in 20 seconds or less.
- 4 = Requires unilateral support (cane, single crutch) to walk; walks 8 meters in 20 seconds or less.
- 5 = Requires bilateral support (canes, crutches, walker) and walks 8 meters in 20 seconds or less; or, requires unilateral support but walks 8 meters in greater than 20 seconds.
- 6 = Requires bilateral support and walks 8 meters in greater than 20 seconds. May use wheelchair on occasion.
- 7 = Walking limited to several steps with bilateral support; unable to walk 8 meters. May use wheelchair for most activities.
- 8 = Restricted to wheelchair; able to transfer independently.
- 9 = Restricted to wheelchair; unable to transfer independently.

Appendix C: Standard Neurological Status (SNS)

APPENDIX C

Standardized Neurologic Status (SNS)

		Total Score	
I. Definite Supraspinal Signs			
<input type="checkbox"/>	Vision (right) (quantitative)	0 = ≥ 1.0 1 = ≤ 0.9 2 = ≤ 0.7	
<input type="checkbox"/>	Vision (left) (quantitative)	3 = < 0.5 4 = ≤ 0.3 5 = ≤ 0.1	
<input type="checkbox"/>	Ocular motor nerve palsy/Gaze palsy	0 = absent 1 = in 1 line of vision 2 = in > 1 line of vision	
<input type="checkbox"/>	Spontaneous nystagmus	0 = absent 1 = present	
<input type="checkbox"/>	Gaze evoked nystagmus	0 = absent 1 = present	
<input type="checkbox"/>	Dissociated nystagmus/INOP	0 = absent 1 = present	
<input type="checkbox"/>	Speech impairment (bulbar/cerebellar)	0 = absent 1 = mild 2 = speech only intelligible with great effort	
<input type="checkbox"/>	Swallowing	0 = not impaired 2 = slightly impaired 4 = frequent deglutition disturbances/coughing	
<input type="checkbox"/>	Breathing	0 = not impaired 6 = impaired	
<input type="checkbox"/>	Intention tremor/nonparetic dysmetria	0 = absent 1 = mild (motor response not impaired) 2 = moderate (motor response noticeably impaired) 3 = severe (e.g., hands can no longer be used)	
<input type="checkbox"/>	Truncal ataxia	0 = absent 2 = mild 4 = moderate (unaided sitting with arms stretched out just barely possible) 8 = severe (unaided sitting no longer possible)	
<input type="checkbox"/>	Ataxic gait	0 = absent 8 = present	
<input type="checkbox"/>	Affect	0 = not impaired 1 = impaired	
<input type="checkbox"/>	Drive/motivation	0 = not impaired 1 = slightly impaired 3 = severely impaired	
<input type="checkbox"/>	Psycho-motor slowing		
II. Paresis			
<input type="checkbox"/>	Right arm/hand	5 = no muscle activity 4 = visible muscle contraction without movement	
<input type="checkbox"/>	Left arm/hand		
<input type="checkbox"/>	Right leg/foot	3 = locomotive effect seen when at rest 2 = motion possible against no resistance 1 = motion possible against moderate resistance 0 = normal muscle strength	
<input type="checkbox"/>	Left leg/foot		
III. Spasticity			
<input type="checkbox"/>	Right arm	0 = absent 1 = increase in tone may be overcome in rapid continuous movement 2 = spasticity may be overcome only very slowly and with great effort	
<input type="checkbox"/>	Left arm		
<input type="checkbox"/>	Right leg		
<input type="checkbox"/>	Left leg		
IV. Sensation			
<input type="checkbox"/>	Abdomen	<u>Sense of touch:</u> 0 = not impaired 1 = impaired	
<input type="checkbox"/>	Right arm/hand		
<input type="checkbox"/>	Left arm/hand		
<input type="checkbox"/>	Right leg/foot		
<input type="checkbox"/>	Left leg/foot		
<input type="checkbox"/>	Right malleolus ext.	<u>Sensitivity to vibrations:</u> 0 = > 6/8 1 = $\leq 6/8$ 2 = $\leq 3/8$	
<input type="checkbox"/>	Left malleolus ext.		
V. Urinary Bladder Impairment			
<input type="checkbox"/>	Urgency	0 = absent 1 = present	
<input type="checkbox"/>	Urinary incontinence	0 = absent 1 = rarely 3 = about weekly 4 = severe (diapers or urinal required)	
<input type="checkbox"/>	Overflow incontinence (Ischuria paradoxa)	0 = absent 1 = present	
<input type="checkbox"/>	Indwelling catheter/suprapubic drain	0 = absent 1 = present	
<input type="checkbox"/>	Residual urine	0 = ≤ 50 ml 1 = < 100 ml 2 = ≥ 100 ml x = not done	

**Appendix D: Summary of Preclinical Studies in Experimental Allergic
Encephalomyelitis (EAE)**

APPENDIX D

Summary of Preclinical Studies in Experimental Allergic Encephalomyelitis (EAE)

Mitoxantrone has been shown to be effective in a variety of EAE models. Major findings from the studies of mitoxantrone in EAE are summarized in the table that follows.

Results obtained using active models of EAE are categorized as prophylactic effects (mitoxantrone treatment beginning at the time of immunization), effector-level inhibition (treatment beginning after effector cells are primed but before disease onset is evident), or therapeutic protection (treatment beginning after disease has been clearly established).

Summary of EAE Study Results

Study	Type of EAE model	Mitoxantrone treatment regimen	Results
<i>Therapeutic Protection in Active EAE Models</i>			
Levine, 1985	Relapsing disease Lewis rats	1 mg/kg IP or SC (leg) Single dose Day 14	All 6 control rats relapsed. Mitox prevented relapse in 12 of 14 rats.
Watson, 1991	Relapsing disease Biozzi AB/H mice	2.5 mg/kg IP, twice weekly Days 27-40	Mitox prevented relapse in 12/13 mice (p < 0.002).
<i>Effector-Level Inhibition in Active EAE Models</i>			
Ridge, 1985	Acute disease Lewis rats	0.25 – 0.5 mg/kg/day IP Days 7-16 Compared to 1.25 – 5.0 mg/kg CP	Mitox at 0.25 – 0.5 mg/kg suppressed clinical and histological lesions (p < 0.05).
Watson, 1991	Acute disease Biozzi AB/H mice	1 – 2.5 mg/kg/day IP Days 12-19	Mitox at 2.5 mg/kg prevented development of acute disease.
Baker, 1992	Acute disease Biozzi AB/H mice	0.5 – 5.0 mg/kg IP Single dose Day 9	A single dose of 5 mg/kg Mitox completely inhibited the development of EAE.
<i>Prophylactic Effects in Active EAE Models</i>			
Ridge, 1985	Acute disease Lewis rats	0.125 – 0.5 mg/kg/day IP for 16 days starting on Day 0. Compared to 1.25 – 5.0 mg/kg	Mitox at 0.25 – 0.5 mg/kg suppressed clinical and histological lesions (p < 0.05).
Lublin, 1987	Acute disease (SJL/J x BALB/c) F1 mice	0.25 or 0.5 mg/kg/day IP 10 days starting on Day 0	Mitox prevented the development of EAE.
Lublin, 1987	Delayed onset disease SJL/J mice	0.05 mg/kg IP 3x/week for 12 weeks.	Mitox significantly delayed the onset of disease.
Mustafa, 1993	Acute disease Lewis rats	0.5 mg/kg IP, alternate days (Days 0-15). Compared to CsA at 3.0 or 20 mg/kg, alternate days (Days 0-22)	Mitox completely protected rats from EAE.
<i>Treatment of Passive EAE</i>			
Ridge, 1985	Lewis rats immunized with sensitized, syngeneic immune cells	<ul style="list-style-type: none"> • Donors: 0.5 mg/kg/day x 14 days • Cells in vitro: 0.01-0.001 mg/mL • Recipient pretreatment: 0.5 mg/kg/day IP x 5 days 	Mitox prevented the development of passive EAE in all three experimental designs.

Mitox = mitoxantrone; CP = cyclophosphamide; CsA = cyclosporine A.

References for EAE Study

Baker D, O'Neill JK, Davison AN, et al. Control of immune-mediated disease of the central nervous system requires the use of a neuroactive agent: elucidation by the action of mitoxantrone. *Clin Exp Immunol* 1992;90:124-128.

Levine S, Saltzman A. Regional suppression, therapy after onset and prevention of relapses in experimental allergic encephalomyelitis by mitoxantrone. *J Neuroimmunol* 1985;13:175-181.

Lublin FD, Lavassa M, Viti C, et al. Suppression of acute and relapsing experimental allergic encephalomyelitis with mitoxantrone. *Clin Immunol and Immunopathol* 1987;45:122-128.

Mustafa MD, Diener P, Sun JB, et al. Immunopharmacologic modulation of experimental allergic encephalomyelitis: low-dose cyclosporin-A treatment causes disease relapse and increased systemic T and B cell-mediated myelin-directed autoimmunity. *Scand J Immunol* 1993;38:499-507.

Ridge SC, Sloboda AE, McReynolds RA, et al. Suppression of experimental allergic encephalomyelitis by mitoxantrone. *Clin Immunol Immunopathol* 1985;35:35-42.

Watson CM, Davison AN, Baker D, et al. Suppression of demyelination by mitoxantrone. *Int J Immunopharmacol* 1991;13:923-930.

**Appendix E: Statistical Methodology for the Primary Efficacy Analysis
in Study 901**

APPENDIX E

Statistical Methodology for the Primary Efficacy Analysis in Study 901

Three methodological approaches can be used for clinical studies with multiple primary endpoints to produce a statistical analysis that protects the experiment-wise error rate:

1. Combine the attributes of all primary endpoints into a single composite endpoint and perform an analysis of this composite endpoint using the predefined alpha level.
2. Adjust the alpha level, usually by a Bonferroni adjustment, and perform separate analyses of each primary endpoint using the adjusted alpha level.
3. Perform a multivariate analysis using the predefined alpha level.

For Study 901, there was no generally acceptable composite score that could be adopted. Further, a Bonferroni adjustment was impractical due to the large number of endpoints. With the approval of the German regulatory agency BfArM, a nonparametric multivariate statistical analysis was specified in the protocol to establish primary efficacy results. This statistical methodology, described below, has been published in preeminent American statistical journals, and shown to be statistically valid. Validated, commercially available software (SmarTest 1995) has been developed to perform this analysis. The FDA has been provided with a copy of this software, along with documentation and validation information, for review.

The five primary efficacy variables for Study 901 were:

- Change in EDSS at 24 months relative to baseline value.
- Change in AI at 24 months relative to baseline value.
- Number of relapses requiring corticosteroid treatment.
- Time to the first relapse requiring corticosteroid treatment.

- Change in SNS score at 24 months relative to baseline value.

These efficacy variables were tested in one combined hypothesis of stochastic ordered alternatives by the generalized Wilcoxon-Mann-Whitney (Wei-Lachin) procedure (Lachin 1992, Wei 1984). The test was to be conducted for a one-sided hypothesis with $\alpha = 0.05$ for the difference between the mitoxantrone 12 mg/m² treatment group and placebo group. This is a nonparametric global test, hypothesizing that the differences between groups are all in the same direction; i.e., one group is superior in at least one of the variables.

The hypotheses stated in this test are:

$$H_0 : \Theta_k = 0 \quad \text{for all } k=1,2,3,4,5 \quad (\text{variables tested})$$

$$H_1 : \Theta_k \geq 0 \quad \text{for all } k=1,2,3,4,5 \quad \text{with } \Theta_k > 0 \text{ for at least one } k.$$

The Θ_k are the estimators of the difference between the two samples, the Mann-Whitney differences. A Mann-Whitney difference is defined as the difference between (1) the probability that a member of sample 1 has a larger value for the variable of interest than a member of sample 2 and (2) the probability that a member of sample 2 has a larger value for the variable of interest than a member of sample 1.

The Mann-Whitney difference is also a valid estimator for variables with missing values or censored observations such as the variable “time to first relapse requiring treatment.”

The test statistic Z derived from the Wei-Lachin procedure has an asymptotic normal distribution and is defined as:

$$Z = (J' \Theta) / [J' \Sigma J]^{1/2}$$

with Σ being the covariance-matrix of Θ and $J'=(1,\dots,1)$, a vector of weights one.

This test statistic is the nonparametric equivalent to Hotelling’s one-sided parametric T² test. If the test resulted in a significant p-value, all five single criteria were to be tested

univariately with $\alpha = 0.05$ in sequence according to the principle of a priori ordered hypotheses (Maurer 1995). The sequence of testing was revised by Amendment 3 to be EDSS, AI, number of relapses requiring corticosteroid treatment, time to the first relapse requiring such treatment, and SNS. In this document, results of two-sided tests are reported with $\alpha = 0.05$.

References

Lachin JM. Some large-sample distribution-free estimators and tests for multivariate partially incomplete data from two populations. *Stat Med* 1992; 11:1151-70.

Maurer W, Hothorn LA, Lehmacher W. Multiple comparisons in drug clinical trials and preclinical assays: A-priori ordered hypothesis. In: *Biometrie in der chemisch-pharmazeutischen industrie 6: Testing principles in clinical and preclinical trials* (Vollmar J, ed.). Gustav Fischer Verlag, Stuttgart 1995:3-18.

SmarTest Handbook Version 1.2, idv – Datenanalyse und Versuchspanung, Gauting – Germany, 1995.

Wei LJ, Lachin JM. Two-sample asymptotically distribution-free tests for incomplete multivariate observations. *J Am Stat Assoc* 1984; 79(387):653-61.

Appendix F: Summary of Sensitivity Analyses Conducted in Study 901

APPENDIX F

Summary of Sensitivity Analyses Conducted in Study 901

Post-hoc sensitivity analyses were performed on various patient subgroups. This summary provides data from the mitoxantrone 12 mg/m² and placebo groups only and is limited to the analysis of the primary efficacy variables. These analyses were consistent with the efficacy results seen in the population as a whole and confirmed the superiority of mitoxantrone compared to placebo.

Gender Effect

Analysis of Gender Effect

Endpoint	Female		Male	
	Placebo N = 31	Mitox 12 mg/m ² N = 28	Placebo N = 33	Mitox 12 mg/m ² N = 32
EDSS change (mean, M24 minus baseline)*	0.08	-0.46	0.36	0.16
AI change (mean, M24 minus baseline)*	0.61	0	0.91	0.56
SNS change (mean, M24 minus baseline)*	1.29	-2.50	0.27	0.19
No. treated relapses (total, adjusted for early withdrawals)	43.59	6.28	33.18	17.80
Time to 1 st relapse requiring treatment (median months)	11.10	NR	NR	NR

Mitox = mitoxantrone; NR = median not reached within 24 months.

* Negative values indicate improvement for this scale, higher positive values indicate worsening.

Age Effect

Patients were evaluated based on a age cutoff of 40, median age for this study population.

Analysis of Age Effect

Endpoint	< 40 Years		≥ 40 Years	
	Placebo N = 32	Mitox 12 mg/m ² N = 31	Placebo N = 32	Mitox 12 mg/m ² N = 29
EDSS change (mean, M24 minus baseline)*	-0.03	-0.37	0.48	0.12
AI change (mean, M24 minus baseline)*	0.72	0.06	0.81	0.55
SNS change (mean, M24 minus baseline)*	-0.13	-1.58	1.66	-0.52
No. treated relapses (total, adjusted for early withdrawals)	38.74	14.08	38.03	10.00
Time to 1 st relapse requiring treatment (median months)	15.01	NR	14.19	NR

Mitox = mitoxantrone; NR = median not reached within 24 months.

* Negative values indicate improvement for this scale, higher positive values indicate worsening.

Type of MS

Patients were evaluated based on the type of MS as defined in the protocol.

Analysis Based on Type of MS

Endpoint	Remitting Progressive		Secondary Progressive	
	Placebo N = 10	Mitox 12 mg/m ² N = 17	Placebo N = 23	Mitox 12 mg/m ² N = 15
EDSS change (mean, M24 minus baseline)*	0.09	-0.30	0.34	0.02
AI change (mean, M24 minus baseline)*	0.41	0.14	1.06	0.44
SNS change (mean, M24 minus baseline)*	-0.31	-2.46	1.66	0.16
No. treated relapses (total, adjusted for early withdrawals)	45.61	8.61	31.17	15.47
Time to 1 st relapse requiring treatment (median months)	11.10	NR	21.95	NR

Mitox = mitoxantrone; NR = median not reached within 24 months.

* Negative values indicate improvement for this scale, higher positive values indicate worsening.

Relapse 1 Year Prior to Study

Patients were evaluated based on whether they had a history of relapse(s) in the year preceding enrollment or not.

Relapse 1 Year Prior to Study

Endpoint	Yes		No	
	Placebo N = 22	Mitox 12 mg/m ² N = 25	Placebo N = 11	Mitox 12 mg/m ² N = 7
EDSS change (mean, M24 minus baseline)*	0.05	-0.22	0.67	0.13
AI change (mean, M24 minus baseline)*	0.61	0.20	1.17	0.60
SNS change (mean, M24 minus baseline)*	-0.74	-1.78	4.61	2.33
No. treated relapses (total, adjusted for early withdrawals)	58.81	20.34	17.96	8.78
Time to 1 st relapse requiring treatment (median months)	13.83	25.30	15.38	NR

Mitox = mitoxantrone; NR = median not reached within 24 months.

* Negative values indicate improvement for this scale, higher positive values indicate worsening.

Study Completers

Patients were evaluated based on whether they completed all eight course of study drug (completers) or not (i.e., intent-to-treat group)

Study Completers

Endpoint	Completers		Intent to Treat	
	Placebo N = 23	Mitox 12 mg/m ² N = 24	Placebo N = 33	Mitox 12 mg/m ² N = 32
EDSS change (mean, M24 minus baseline)*	0.14	-0.25	0.23	-0.13
AI change (mean, M24 minus baseline)*	0.66	0.25	0.77	0.30
SNS change (mean, M24 minus baseline)*	0.60	-0.88	0.77	-1.07
No. treated relapses (total, adjusted for early withdrawals)	46.33	13.23	76.77	24.08
Time to 1 st relapse requiring treatment (median months)	16.20	25.30	14.2	NR

Mitox = mitoxantrone; NR = median not reached within 24 months.

* Negative values indicate improvement for this scale, higher positive values indicate worsening.

APPENDIX G

Published Papers

This appendix contains reprints of the following papers:

Bastianello S, Pozzilli C, D'Andrea F, et al. A controlled trial of mitoxantrone in multiple sclerosis: Serial MRI evaluation at one year. *Can J Neurol Sci* 1994; 21:266-70.

De Castro S, Cartoni D, Millefiorini E, et al. Noninvasive assessment of mitoxantrone cardiotoxicity in relapsing remitting multiple sclerosis. *J Clin Pharmacol* 1995; 35:627-32.

Edan G, Miller D, Clanet M, et al. Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: A randomized multicentre study of active disease using MRI and clinical criteria. *J Neurol Neurosurg Psychiatry* 1997;62:112-8.

Hartung H-P, Gonsette R, MIMS Study Group. Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, randomized, observer-blind phase III trial: clinical results and three-year follow-up. *Neurology* 1999a;52(Suppl 2):A290. (Abstract S45.005).

Hartung H-P, Gonsette R, MIMS Study Group. Mitoxantrone in progressive multiple sclerosis (MS): Clinical results and three-year follow-up of the MIMS trial. *Mult Scler* 1999;5(Suppl 1):S15. (Abstract 56).

Hartung H, Gonsette R, MIMS Study Group. Mitoxantrone in progressive multiple sclerosis (MS): A placebo-controlled, randomized, observer-blind European phase III multicenter study -- Clinical results. *Mult Scler* 1998;4:98. (Abstract 207).

Krapf H, Morrissey SP, Zenker O, et al. Mitoxantrone in progressive multiple sclerosis (MS): A placebo-controlled, randomized, observer-blind European phase III multicenter study -- MRI results. *Mult Scler* 1998;4:380. (Abstract P3024).

Millefiorini E, Gasperini C, Pozzilli C, et al. Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome. *J Neurol* 1997;244:153-9.

Appendix G: Published Papers

Bastianello S, Pozzilli C, Millefiorini E, Trojano M, et al. 1994

A Controlled Trial of Mitoxantrone in Multiple Sclerosis: Serial MRI Evaluation at One Year

S. Bastianello, C. Pozzilli, F. D'Andrea, E. Millefiorini, M. Trojano, S. Morino, C. Gasperini, A. Bozzao, M. Gallucci, C. Andreula, L. Bozzao, D. Gambi and M. Prencipe

Abstract: We present the results of a randomized double-blinded placebo controlled, multicenter trial of low-dose mitoxantrone (MX), after one year, in 25 patients with relapsing-remitting multiple sclerosis, who had serial enhanced magnetic resonance imaging (MRI). Treatment groups were balanced for age, gender, duration of illness and neurological disability. Five of the 13 MX patients and 10 of the 12 placebo patients had exacerbations during treatment ($p < 0.02$). The mean change in the extended disability status scale was not significantly different between the MX and placebo treatment groups. Serial Gadolinium-DTPA enhanced MRI detected no significant difference between the MX treated and placebo groups in the mean total number of new, enlarging, or Gadolinium-DTPA enhancing lesions; there was a trend toward a reduction of new, enlarging and Gadolinium-DTPA enhancing lesions in MX patients. Despite this ameliorating effect, the results indicate that serial Gadolinium-DTPA enhanced MRI performed over one year in a limited number of patients, could not provide conclusive evidence for a role of MX therapy in relapsing-remitting multiple sclerosis.

Résumé: Étude contrôlée du mitoxantrone dans la sclérose en plaques: évaluation sériée par RMN à un an. Nous présentons les résultats à un an d'une étude multicentres, à double insu, contrôlée par placebo, du mitoxantrone (MX) à faible dose chez 25 patients atteints de sclérose en plaques (SEP) évoluant par poussées et rémissions, qui ont subi une évaluation sériée par RMN renforcé. Les groupes étaient équilibrés pour l'âge, le sexe, la durée de la maladie et l'ancienneté neurologique. Cinq des 13 patients sous MX et 10 des 12 patients sous placebo ont eu des poussées sous traitement ($p < 0.02$). Le changement moyen à l'échelle élargie d'évaluation de l'invalidité n'était pas significativement différent entre les groupes. Le RMN sérié renforcé, au Gadolinium-DTPA, n'a pas détecté de différence significative entre le groupe traité au MX et le groupe placebo quant au nombre total moyen de lésions nouvelles, en expansion ou rehaussantes; il y avait une tendance à la diminution des lésions nouvelles, en expansion et rehaussantes chez les patients sous MX. En dépit de cette amélioration, les résultats indiquent que l'évaluation sériée, par RMN renforcé, au Gadolinium-DTPA, faite sur une période d'un an chez un petit nombre de patients, n'a pu apporter de preuve concluante d'un rôle du traitement par le MX dans la SEP évoluant par poussées et rémissions.

Can. J. Neurol. Sci. 1994; 21: 266-270

Besides the well-established diagnostic value of magnetic resonance imaging (MRI) in multiple sclerosis (MS),^{1,2} recent studies suggest that it reveals the presence of disease activity as measured by new abnormalities on T2 weighted images or by gadolinium enhancing lesions in patients who are clinically stable.³⁻¹¹ Thus, MRI may provide a suitable tool for assessing the effectiveness of clinical therapeutical trials.¹⁰⁻¹⁴ The useful effect of treatment should be observed by MRI in a limited number of patients and after a shorter period of time than that required by clinical monitoring alone.¹³ Some limited benefits of immunosuppressant and immunomodulatory drugs have been reported in the treatment of MS. One recent approach has been the use of mitoxantrone (MX) as a therapeutic agent.

Mitoxantrone is an antineoplastic agent which intercalates into DNA and exerts a potent suppressive influence upon the humoral immune response.¹⁵⁻¹⁷

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Preliminary open-label trials to assess the potential efficacy of treatment with MX have been performed only in progressive MS,¹⁸⁻²⁰ suggesting that MX did not completely suppress clinical and MRI evidence of ongoing disease.²¹ Recent data, however, indicate that therapy may be more effective if used early when there is less neurological damage and when the demyelinating process is just beginning.²² On the basis of these assumptions, we started a 2-year, randomised double-blinded, placebo controlled, multicenter trial of MX in relapsing-remitting MS patients to determine the clinical efficacy of this therapy. We now present the preliminary results after 1-year treatment in a subgroup of patients who underwent serial MRI evaluation.

SUBJECTS AND METHODS

The 2-year randomized, double-blinded, placebo controlled multicenter trial was conducted at seven Italian centers: Universities of Bari, Catanzaro, Chieti, Napoli, Roma, Siena, and L'Aquila, the latter also being the coordinating center.

The subgroup of patients which underwent serial MRI evaluation was selected from four centers (Universities of Bari, Chieti, Roma and L'Aquila) and referred to L'Aquila University in order to perform sequential scans.

The trial design was approved by Internal Review Boards and by the National Health Service.

Patient enrolment and pre-trial observation

Inclusion criteria were: a definite diagnosis of MS;²³ a relapsing-remitting disease course, defined as two or more relapses occurring in the 24 months prior to study entry; age between 18 and 45 years; disease duration from 1 to 10 years; disability no less than 2 or more than 5 on the Kurtzke Expanded Disability Status Scale (EDSS).²⁴

We excluded patients who were HIV-positive, with previous cardiovascular disease, with left ventricular ejection fraction of less than 50% as determined by echocardiography, subjects presenting renal, liver and/or respiratory dysfunctions, diabetes, malignancy, psychiatric illness, pregnancy and women not practicing contraception, as well as patients who had taken previous immunosuppressant medications (such as azathioprine, cyclophosphamide, plasmapheresis) or were taking steroids during the 3 months before entry. Finally, patients incapable of fulfilling the requirements of the study or signing the informed consent were also excluded.

Study design and data collection

When a patient became eligible, the investigators notified the relevant center which validated the eligibility of the patient and assigned a randomisation code number.

For determination of sample size, it was assumed to be important to detect a 1 point difference in the mean change from baseline of the EDSS in 25% of the MX treated group relative to 50% of the placebo treated group at the time of scheduled efficacy analysis. According to this design and with an alpha error = 0.05 and beta error = 0.20, the required number of patients to achieve statistical significance was 45 patients per arm. Up to June 1993, 52 patients had been enrolled into the study.

After examining clinical and MRI data at 1-year, the code was broken by two not blinded investigators (BS and PC);

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therefore the blindness of the second year of the study was maintained.

Of the total patients who were randomised in the trial, 25 (screened between January 1991 and December 1992) were enrolled in the present 1-year serial MRI follow-up study.

The subjects (10 men and 15 women) were randomly assigned to a recipient group that either received MX (n = 13) or a placebo (n = 12). Randomisation for both groups was performed simultaneously.

Treatment

Patients assigned to immunosuppressive treatment received a 30 minute infusion of MX intravenously (8 mg/m²) every month for 1 year; the intravenous bag and tubing were black to ensure patients blinding. Placebo group patients received a solution containing the vehicle alone.

Blood and urine samples and ECG were carried out upon entry to the trial and at each visit. Complete blood counts were obtained from each patient every two weeks. Echocardiography at 0, 6 and 12 months was performed in order to assess the potential cardiac toxicity.

Other drugs were allowed during the trial such as cholinergic and spasmolytic drugs or short courses of steroids (high dose intravenous methylprednisolone 1 g day for 6 days) for relapses.

Evaluation of patients

All patients were examined by four blinded neurologists at each center. Neurological examination was undertaken by means of the EDSS prior to starting therapy and at 12-months. Primary clinical end-points were considered the change in EDSS and the number of exacerbations experienced during the follow-up.

We defined as clinical worsening an increase > 1 point on the EDSS.

Exacerbation was defined as the appearance of new symptom or worsening of an old one, attributable to MS and lasting at least 24 hours in absence of fever. Participating neurologists were trained in the application of the EDSS during a joint session which included repeated rating of patients with MS who have varying levels of disability.

MRI assessment

MRI examinations, performed at 0, 2, 4, 6 and 12 months, were obtained with a 0.2 Tesla permanent unit, using T2 spin echo sequences on axial plane; the enhanced study performed after Gd-DTPA administration (0.1 mmol/kg) was undertaken using T1 weighted sequences on the same axial planes. Slices with 7.5 mm thickness without gap between sections were obtained for all the sequences. In order to obtain comparable examinations during the follow-up scans, a midline sagittal scout slice was always performed at the beginning of the study. In this way we oriented axial sections on the same horizontal plane along a line passing through the basis of the frontal lobe and the caudal portion of quadrigeminal plate.⁸

Image evaluation

MRI data were analysed by two blinded neuroradiologist (BS, BA) and questionable lesions were reviewed by another neuroradiologist as supervisor (BL). Prior to the study, the neuroradiologists were trained to minimize inter-observer differences.

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variability in establishing when lesions first appear, changes in size and enhancement. The inter- and intra-rater variability during serial examinations was less than 5%. Demyelinating areas, seen on T2 weighted images, and Gd-DTPA enhancement seen on T1 weighted images, were detected at the initial MRI study. Follow-up T2 and T1 weighted scans were sequentially analysed for the presence of new disease activity. Three types of "active" lesions were identified: 1) new lesions; 2) lesions which subsequently enlarge; a change exceeding more than 33% (1/3) should be required; 3) enhancing lesions.

Statistical analysis

Differences between means and mean changes were tested using the Student's 2-sample t test, and differences between proportions were tested using the chi-squared test. The Spearman Rank correlation coefficient was used to compare changes in EDSS score and the total number of new, enlarging and enhancing MRI lesions at 1-year follow-up.

RESULTS

The clinical and MRI characteristics of the 25 patients included in the study are shown in Table 1; they were balanced for age, gender, duration of illness and neurological disability. All patients completed the entire treatment being able to tolerate the medication; adverse reactions were generally mild and readily treated. Seven patients reported nausea, 2 patients experienced amenorrhoea which resolved rapidly with cessation of therapy and 1 patient had diarrhoea, vomiting and low grade fever. Side effects due to contrast media were not observed.

There was a statistically significant difference in the mean exacerbation rate and number of patients exacerbating during

the study favouring MX (Table 2). However, no statistical difference was observed in mean EDSS change at 1-year and in the proportion of patients with EDSS deterioration; a worsening in EDSS (1 point or more) was seen in 1 (8%) MX treated patient and in 2 (17%) cases of the placebo group (p = 0.49).

The number of patients showing new, enlarging and Gd-DTPA enhancing lesions during 1 year follow-up study are reported in Figure 1. New, enlarging and enhancing lesions were detected respectively in 11 (85%), 5 (38%) and 4 (31%) patients treated with MX and in 11 (92%), 8 (67%) and 7 (58%) patients of the placebo group.

Mean number of new, enlarging and Gd-DTPA enhancing lesions at 2, 4, 6 and 12 months for both treatment groups are shown in Table 3. There were no significant differences in the mean number of lesions/patient between the two groups at each serial examination. In the MX group, however, a trend was noted towards a total reduction of new (MX 2.30, placebo 3.91; p < 0.27), enlarging (MX 1, placebo 1.83; p < 0.42) and Gd-DTPA

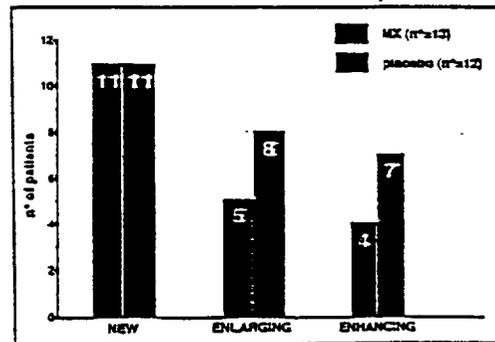


Figure 1. Number of patients showing new, enlarging and Gd-DTPA enhancing lesions during 1-year follow-up.

Table 1: Baseline clinical and MRI characteristics of patients entering treatment.

	MX	Placebo	Statistical test/p *
No. of patients (male/female)	13 (5/8)	12 (5/7)	Chi.sq/0.37
Age	29.9 (5.2)*	28.5 (6.5)	t test/0.55
Age at onset (yrs)	23.7 (5.6)	24.3 (5.3)	t test/0.31
Duration (yrs)	5.2 (2.4)	5 (2.7)	t test/0.86
Exacerbations in prior 2 years	2.3 (1.2)	3.3 (1.2)	t test/0.25
EDSS	3.7 (0.7)	3.5 (1.0)	t test/0.49
No. of lesions	25.5 (21.7)	23 (24.9)	t test/0.73
No. of enhancing lesions	0.3 (0.5)	0.5 (0.9)	t test/0.51

* All t tests are two tailed
 * Mean (SD)
 MX Mitoxantrone
 EDSS Expanded Disability Status Scale score

Table 2: Treatment groups after 1-year follow-up.

	MX (13)	Placebo (12)	Statistical test/p *
Mean exacerbation rate	0.54 (0.9)*	1.67 (1.2)	t test/0.014
No. of patients exacerbating	5 (38%)	10 (83%)	Chi.sq/0.02
Mean change in EDSS **	-0.27 (0.7)	-0.08 (0.6)	t test/0.18
Proportion of patients with EDSS deterioration ***	8%	17%	Chi.sq/0.49

* All t tests are two tailed
 * Mean (SD)
 ** EDSS Expanded Disability Status Scale score (= indicate a worsening at the end of treatment).
 *** A worsening >1 point on the EDSS.

Table 3: Mean number of new, enlarging and Gd-DTPA enhancing lesions/patient at serial MRI examinations for both treatment groups.

Months	MX			Placebo		
	New	Enlarging	Enhancing	New	Enlarging	Enhancing
0-2	0.61 (0.8)*	0.23 (0.6)	0.31 (0.6)	0.58 (0.8)	0.50 (0.8)	0.33 (0.5)
2-4	0.23 (0.6)	0.31 (0.8)	0	0.91 (1.7)	0.25 (0.6)	0.50 (1.4)
4-6	0.23 (0.6)	0.31 (0.9)	0.08 (0.3)	0.50 (1.0)	0.25 (0.6)	0.25 (0.5)
6-12	1.23 (1.7)	0.15 (0.4)	0.08 (0.3)	1.92 (2.5)	0.83 (2.0)	0.08 (0.3)
Total	2.30 (2.1) [2]**	1 (1.8) [0]	0.46 (0.7) [0]	3.91 (4.1) [2.5]	1.83 (3.0) [1]	1.16 (1.5) [1]

* Mean (SD)
 ** Median

enhancing lesions (MX 0.46, placebo 1.16; $p < 0.13$). This reduction was of 41%, 45% and 60% for new, enlarging and enhancing respectively.

Finally, there were no significant relationships between changes in the EDSS score and the total number of new, enlarging and Gd-DTPA enhancing MRI lesions at 1 year follow-up in both groups (data not shown).

DISCUSSION

The rationale for immunosuppressive treatment lies in the suppression of the inflammatory reaction of the immune system in order to prevent or to arrest the process of demyelination. Drug treatments with immunosuppressive agents such as azathioprine, cyclophosphamide, cyclosporin or total lymphoid irradiation show only modest therapeutical benefit at safe doses in relapsing-remitting MS.²⁸

Mitoxantrone is a well-known antineoplastic agent with recently detected immunomodulating properties, especially on B-lymphocyte function. Because the clinical tolerance of MX is better than that of other immunosuppressive drugs and long-term toxicity markedly lower, early pilot studies in progressive MS patients suggested that MX should be a candidate for controlled clinical trials.¹⁸⁻²³

The primary purpose of this double-blinded, placebo-controlled trial was to determine whether monthly therapy with MX at a dose of 8 mg/m² every month for 1 year could alter disease progression in relapsing-remitting MS patients.

We found in this 1-year interim analysis a slowing of the clinical progression in MX treated patients compared with placebo as demonstrated by a reduction in the mean exacerbation rate and in the number of patients showing clinical exacerbations (see Table 2). Furthermore, a minor improvement in the mean EDSS score was detected in the MX group but not in the placebo group. However, it has been suggested that change in mean EDSS is clinically irrelevant and methodologically incorrect.²⁷ Differences in the proportion of patients changing by a given degree of disability represent the most feasible endpoint in the context of short-term clinical trials.²⁸ However, because of the small sample and the short-term follow-up period, we could observe a 1 point worsening in EDSS only in 2 (17%) patients receiving placebo and in 1 (8%) of the MX group.

Therefore, for a proper evaluation of the clinical results we must await the end of the multicenter study in the whole randomized sample.

Serial MRI examination has been recently proposed as an effective tool to evaluate the efficacy of short-term therapy.^{10,11,13-14} When measuring therapeutic efficacy by MRI it is necessary to consider several different aspects such as the duration of the study, the number of patients and the frequency of scanning.¹³ The enumeration of new, enlarging and Gd-DTPA enhancing lesions seems to be the most suitable measure of short-term outcome, while lesion/volume measurement is more appropriate for long-term studies.^{13,29}

The marked variation in MRI activity, both between and within patients over time, implied the need to study a substantial number of patients. As recently suggested by McFarland et al.,¹¹ the sample size required to detect a significant reduction in lesion frequency in a therapeutical trial using a parallel group design, closely depends on the number of monthly MRI scans per subject. Monthly examinations for up to six months seem to be the most suitable interval using Gd-DTPA enhanced MRI.^{11,13} With this frequency at least 90 subjects for each of the two groups (treated and placebo) would be required to make the trial design statistically acceptable.¹¹ On the basis of these assumptions the small sample (25 patients) and the frequency of scanning (2, 4, 6 and 12 months), appear to be the major limitations of the present study.

Considering our scan frequency, the likelihood of missing MRI activity appears more related to the detection of the enhanced lesions rather than new and enlarging ones. The duration of enhancement in relapsing-remitting patients is extremely variable ranging from less than 1 month to greater than 12 months;³⁰ generally, however, the enhancing phase disappears within 4 weeks in about 2/3 of lesions.^{4,10} In our placebo group we detected a mean rate of 0.33 Gd-DTPA enhancing lesions/patient every two months, whereas the mean number of enhancing lesions/month observed in previous reports of untreated relapsing-remitting patients, ranges between 1.33 to 3.25.^{4,10,11} Therefore, we might miss an enhancement in at least 50% of lesions with a bimonthly scan interval.

The effect of treatment evaluated by MRI and presented in both Figure 1 and Table 3 shows that no significant differences were observed between the MX and placebo treated patients when examined at 2, 4, 6 and 12 months.

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Although not statistically significant, however, the yearly rate reduction observed in MX patients was of 41%, 45% and 60% for new, enlarging and Gd-DTPA enhancing lesions respectively. These findings are consistent with the significant trend towards a clinical improvement identified in the MX group and need to be confirmed by the end of the multicenter study before a potential benefit of MX can be claimed.

Another interesting point to be discussed is the lack of a significant relationship between change in EDSS score and MRI findings found in both placebo and MX patients. This is far from surprising since clinical and MRI methods measure different aspects of disease activity. Serial MRI studies of relapsing-remitting MS have shown that new abnormalities on the MRI occurred seven times more frequently than clinical events.^{7,10,20} Our results confirm these findings showing a relative stable clinical course demonstrated by low variation of EDSS score after 1 year when compared with MRI activity.

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ADVERSE EFFECTS

Noninvasive Assessment of Mitoxantrone Cardiotoxicity in Relapsing Remitting Multiple Sclerosis

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Multiple sclerosis is the most common cause of neurologic disability in young adults. Recent reports have suggested that Mitoxantrone might be a candidate for clinical trials in multiple sclerosis patients. The authors studied 20 patients with relapsing remitting multiple sclerosis to evaluate cardiac toxicity during a one-year follow-up period. Patients were divided into 2 groups: group A, mitoxantrone treated patients (cumulative dose of 96 mg/m²); group B, placebo patients. The clinical course of multiple sclerosis was assessed using the Expanded Disability Status Scale and the number of relapses during the follow-up. Each patient had an electrocardiogram and a spectral and color flow Doppler echocardiographic examination at enrollment, and 6 and 12 months later, to investigate cardiac toxicity. The mean exacerbation rate was reduced significantly in group A patients. No significant differences in the electrocardiograms or the echocardiographic parameters of systolic and diastolic function were noted between the two groups or in group A during the follow-up. Mitoxantrone treatment seems able to improve the clinical course of relapsing remitting multiple sclerosis patients. It does not show any cardiac toxicity in selected patients at this dosage.

Multiple sclerosis is an inflammatory demyelinating disorder of the central nervous system and is the most common cause of neurological disability in young adults. It is commonly hypothesized that in multiple sclerosis there is (1) an external factor, i.e., a viral infection, that induces (2) an abnormal immunoreaction in subjects with (3) a certain genetic predisposition.^{1,2}

Because of the role of the immune system in multiple sclerosis and because a high exacerbation

rate early in the course of the disease predicts rapid subsequent progression,³ aggressive immunosuppressive therapy may be justified. Recent attempts to improve the course of multiple sclerosis have focused largely on strategies to down-regulate the immune response, using a variety of immunosuppressive agents such as cyclophosphamide,⁴ cyclosporine,⁵ and azathioprine.⁶ High doses of these drugs may have a modest effect on relapse rate and disease progression, but with unacceptable side effects.

Mitoxantrone is a novel anthracenedione antineoplastic agent that intercalates with deoxyribonucleic acid and is a potent inhibitor of both deoxyribonucleic acid and ribonucleic acid synthesis.^{7,8}

Recently, early pilot studies in patients with multiple sclerosis have suggested that mitoxantrone should be a candidate for controlled clinical trials.⁹⁻¹² Because of structural similarity with other anthracyclines, there is the potential risk of mitoxantrone-induced cardiomyopathy. Anthracycline toxicity is irreversible and dose-dependent and is characterized by reduction of left ventricular ejection fraction and congestive heart failure.^{13,14}

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TABLE I
Inclusion and Exclusion Criteria

Inclusion criteria	
Disease	Definite diagnosis of MS according to Poser criteria Relapsing remitting course Two or more relapses occurring in the 24 months before the study entry Duration from 1 to 10 years Disability no less than 2 or no more than 5 on EDSS
Patients	Age between 18 and 45 years Written informed consent before the study entry
Exclusion criteria	
Patients	Positive test for HIV antibodies Heart, renal, lung, or liver disease Psychiatric disease Pregnancy or lactation Known allergy to corticosteroids Other neurological disease
Previous treatments	Corticosteroids during the last 3 months Levamisol, isoprinosin, or plasmapheresis during the last 3 months Interferon Immunosuppressive therapy (azathioprine, cyclosporine, cyclophosphamide, or lymphoid irradiation) during the last 12 months

MS = multiple sclerosis; EDSS = Expanded Disability Status Scale; HIV = human immunodeficiency virus.

The aim of this study was to evaluate the cardiac toxicity, with particular attention to left ventricular systolic and diastolic function, of mitoxantrone in patients with relapsing remitting multiple sclerosis. Clinical efficacy data for mitoxantrone in this disease are included and briefly discussed.

MATERIALS AND METHODS

The authors conducted a prospective, double-blind, placebo-controlled trial of mitoxantrone in 20 patients affected by relapsing remitting multiple sclerosis, diagnosed according to Poser criteria.¹⁵ They studied 10 women and 10 men of mean age 30.9 ± 5.1 years. Inclusion and exclusion criteria are reported in Table I. Patients enrolled were divided into two homogeneous groups for age, Expanded Disability Status Scale (EDSS),¹⁶ disease duration, and number of relapses.

Patients assigned to immunosuppressive treat-

ment (n = 11, group A) received 30 minutes of intravenous infusion of mitoxantrone (8 mg/m²) every month for 1 year (cumulative dose 96 mg/m²). The remaining patients (n = 9, group B), considered the placebo group, received a solution containing the vehicle alone.

Other therapies were allowed during the trial, such as cholinergic or spasmolytic drugs, and high intravenous doses of methylprednisolone (1 g daily for 6 days) were given during a relapse.

All patients had a standard electrocardiogram at enrollment and 4, 8, and 12 months after the beginning of the study. Moreover, to assess the potential cardiac toxicity of mitoxantrone, each patient had an M-mode and two-dimensional echocardiographic study with spectral and color Doppler flow analysis, which was done at enrollment and 6 and 12 months later. The authors used a phased array system (Hewlett-Packard, Andover, MA) with 2.5 and 3.5 MHz transducers. Patients were examined in the left lateral decubitus position, and data were acquired during postexpiratory apnea. The images were recorded on super-VHS videotape and analyzed, independently, by two cardiologists according to the American Society of Echocardiography criteria.¹⁷ Echocardiographic data were calculated as the mean of three measurements with an inter-intraobserver variability that was not statistically significant, as reported.¹⁸

The following parameters were considered: aortic root (Ao) dimension (mm); left atrium (LA) dimension (mm); end-diastolic (DD) and end-systolic (SD) left ventricular dimension (mm); septal wall (SWT) and posterior left ventricular wall (PWT) thickness (mm); left ventricular fractional shortening (FS%), in percentage, calculated using the formula:

$$FS\% = DD - SD / DD \times 100^{19},$$

and left ventricular ejection fraction (EF%), in percentage, calculated using the area-length method.²⁰

Using the spectral Doppler approach, the authors used the following left ventricular time intervals of flow as indices of systolic function²¹: pre-ejection period (PEP), which is the time interval from the onset of electrocardiographic QRS to the onset of aortic systolic flow (ms); left ventricular ejection time (LVET), which is the interval from the onset to the cessation of aortic flow (ms); and ratio of the pre-ejection period to left ventricular ejection time (PEP/LVET).

Diastolic function was assessed by analysis of the following left ventricular inflow Doppler velocities^{22,23}: peak flow velocity in early diastole (V_{maxE}) (cm/s); peak flow velocity in late diastole (V_{maxA}) (cm/s); ratio of the early to late diastolic flow (E/A); and deceleration time (DT), which is the interval re-

ECHOCARDIOGRAPHY AND MITOXANTRONE TOXICITY

TABLE II

Clinical Characteristics

	Age (yr)	Duration of Disease	No. of Relapses		EDSS		P Value
			2 Years Previously	Post	Pre	Post	
Group A	31 ± 5	5.8 ± 1.8	2.82 ± 0.98	0.45 ± 0.52	3.77 ± 0.72	3.42 ± 0.93	NS
Group B	30 ± 4	5.4 ± 2.7	3.00 ± 1.94	1.56 ± 1.01	3.33 ± 0.75	3.44 ± 1.13	NS
	NS	NS	NS	*	NS	NS	

* P = 0.005.

Group A = mitoxantrone-treated patients; Group B = placebo patients; EDSS = Expanded Disability Status Scale; Pre = data at enrollment; post = data at the end of the study; NS = not significant.

quired for the E velocity to decrease from its peak to the baseline (ms).

STATISTICS

Measurements are expressed as mean ± SD. The clinical data for the two groups of patients were compared using an unpaired Student t test. A comparison between data at the beginning and end of the study for groups A and B was made using the paired Student t test. An analysis of variance was used to assess significant differences between the two groups for the echocardiographic data. A P value < .05 was considered statistically significant.

RESULTS

The clinical characteristics of the study population are shown in Table II. No significant differences were present between the treated and placebo groups for age, disease duration, EDSS, and number of relapses occurring in the 24 months before study entry. The mean exacerbation rate was reduced significantly in mitoxantrone treated patients. Although not significant, the authors observed a trend towards a reduced EDSS score in mitoxantrone patients during follow-up.

The electrocardiograms done at trial entry showed no abnormality in all patients but one, who presented with a left bundle branch block. The subsequent electrocardiograms, done at 4, 8, and 12 months from the beginning of the study, showed no modification of wave morphology or appearance of arrhythmia both in patients with normal examinations at entry and in the patient with left bundle branch block.

The M-mode and two-dimensional echocardi-

graphic parameters are reported in Table III. No significant differences in either group were found during the study. Particularly, in group A no differences were observed at 0, 6, and 12 months from the beginning of the study regarding left ventricular systolic function parameters, such as fractional shortening and ejection fraction.

The indices of left ventricular function obtained with spectral Doppler analysis are reported in Table IV. No differences between systolic parameters, such as PEP, LVET, PEP/LVET, or between diastolic indices, such as $V_{max}E$, $V_{max}A$, E/A, and DT were noted for the two groups. Similarly, the authors found no difference between the same parameters in group A patients at 0, 6, and 12 months from the beginning of the study.

DISCUSSION

Classically, multiple sclerosis begins with a relapsing and remitting course. In time, the remissions tend to be less than complete and many patients subsequently pass into a progressive phase, with gradual accumulation of irreversible disability. A smaller number of patients, less than 10%, develop progressive disability from onset without relapses and remissions. Mitoxantrone is a cytotoxic pharmacologic substance of anthracenedionic derivation, generally used as an antineoplastic drug, with an immunosuppressive action. This effect, when used in a limited amounts, markedly influences the humoral immune system, through a direct reduction in B cell numbers.²⁴ *In vivo* and *in vitro* studies indicate that mitoxantrone is an immunomodulator that reduces T cell numbers, suppresses humoral immunity, and causes *in vivo* inhibition of T-helper cell (but not T-suppressor cell) activity.^{24,25}

Recently, mitoxantrone has been tested on relaps-

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TABLE III

M-mode and Two-dimensional Echocardiographic Parameters

	Baseline		6 Months		12 Months		P Value
	Group A	Group B	Group A	Group B	Group A	Group B	
Ao	30 ± 3.1	29 ± 4.9	30 ± 4.0	30 ± 3.3	30 ± 3.4	30 ± 3.3	NS
LA	32 ± 3.9	31 ± 4.2	30 ± 2.7	30 ± 4.0	30 ± 3.2	30 ± 3.6	NS
DD	50 ± 3.8	49 ± 2.0	51 ± 1.9	49 ± 3.8	49 ± 4.1	48 ± 3.4	NS
SD	32 ± 3.1	31 ± 3.1	31 ± 2.1	30 ± 3.5	31 ± 3.9	29 ± 3.5	NS
SWT	7.7 ± 1.4	8.0 ± 1.8	8.0 ± 1.0	7.8 ± 0.8	7.5 ± 1.4	7.4 ± 0.6	NS
PWT	7.5 ± 1.0	7.8 ± 1.2	7.7 ± 0.8	7.8 ± 0.8	7.4 ± 1.0	7.5 ± 0.5	NS
FS	37 ± 2.3	35 ± 3.8	37 ± 2.3	36 ± 2.4	36 ± 3.7	38 ± 4.8	NS
EF	63 ± 5.1	61 ± 5.4	63 ± 3.3	64 ± 3.9	61 ± 1.9	65 ± 3.5	NS

Group A = mitoxantrone-treated patients; Group B = placebo patients; Ao = aortic root dimension (mm); LA = left atrium dimension (mm); DD = left ventricular end-diastolic dimension (mm); SD = left ventricular end-systolic dimension (mm); SWT = septal wall thickness (mm); PWT = posterior left ventricular wall thickness (mm); FS = left ventricular fractional shortening (%); EF = left ventricular ejection fraction (%); NS = not significant.

ing remitting multiple sclerosis patients.¹² In this study, the authors, in a first year interim analysis, evaluated mitoxantrone efficacy in 25 patients, with the disease biological activity assessed using magnetic resonance imaging as the primary endpoint. New, enlarging, or gadolinium-enhancing lesions were detected, respectively, in 11, 5, and 5 patients treated and in 11, 8, and 7 patients of the placebo group. There were no significant differences between the two groups in the mean total number of new, enlarging, and enhancing lesions per patient. However trends towards a reductions in the numbers of new, enlarging, and enhancing lesions in mitoxantrone

patients of 41%, 45%, and 60%, respectively, were found.

The data also show a slowing of multiple sclerosis clinical progression. The authors noted a significant reduction in the mean exacerbation rate of the disease and a trend toward reduced EDSS score in mitoxantrone treated patients compared with the placebo group. Despite the numerous clinical and instrumental studies done to describe and characterize the cardiotoxic effects of mitoxantrone, these still are not clarified completely. One possible explanation is that some of the authors tried to link the characteristics of this drug with those of the anthracycline an-

TABLE IV

Doppler Indices of Left Ventricular Function

	Baseline		6 Months		12 Months		P Value
	Group A	Group B	Group A	Group B	Group A	Group B	
HR	74 ± 8	73 ± 6	75 ± 9	73 ± 7	73 ± 9	74 ± 9	NS
PEP	104 ± 10	107 ± 12	10 ± 17	103 ± 11	109 ± 9	105 ± 12	NS
LVET	284 ± 18	281 ± 22	276 ± 22	268 ± 32	277 ± 19	267 ± 33	NS
PEP/LVET	0.36 ± 0.06	0.33 ± 0.3	0.38 ± 0.06	0.36 ± 0.11	0.39 ± 0.02	0.34 ± 0.07	NS
V _{max} E	71 ± 12	75 ± 8	69 ± 16	72 ± 10	70 ± 14	74 ± 19	NS
V _{max} A	54 ± 10	52 ± 11	50 ± 7	50 ± 14	49 ± 5	50 ± 10	NS
E/A	1.35 ± 0.2	1.43 ± 0.3	1.40 ± 0.4	1.42 ± 0.2	1.44 ± 0.46	1.46 ± 0.2	NS
DT	198 ± 31	202 ± 36	200 ± 33	202 ± 28	206 ± 31	197 ± 31	NS

Group A = mitoxantrone-treated patients; Group B = placebo patients; HR = heart rate (beats/min); PEP = left ventricular pre-ejection period (ms); LVET = left ventricular ejection time (ms); V_{max}E = early diastolic peak left ventricular inflow velocity (cm/s); V_{max}A = late diastolic peak left ventricular inflow velocity (cm/s); DT = early diastolic flow deceleration time (ms); NS = not significant.

ECHOCARDIOGRAPHY AND MITOXANTRONE TOXICITY

tibiotic, for which the cardiotoxicity is well known. Nevertheless, mitoxantrone is clearly not an antibiotic, because it is extracted synthetically. The only resemblance between it and the anthracyclines is the presence of an anthracyclenic nucleus formed by an anthraquinonic point.

It is not clear to what extent this structure makes the mitoxantrone toxicity effects similar to anthracyclenic antibiotics or whether these drugs must be considered substantially different, the similarities seen as purely incidental.¹³

In fact, Crossley²⁶ compared two groups of patients treated with mitoxantrone and noted that the main risk factor in the development of congestive heart failure and reduction of left ventricular ejection fraction was that of previous anthracycline treatment. An analysis of cardiotoxic events done by Dukart and Barone on a population of 3200 mitoxantrone treated patients lead to similar conclusions.²⁷ Leaving out the patients previously exposed to anthracyclenic therapy or to mediastinal radiation or with a significant pre-existent cardiac disease, only 5 of the remaining 86 episodes were related to mitoxantrone. Among those, two patients presented with congestive heart failure with a cumulative dose higher than 240 mg/m². In these two patients, an intramyocardial biopsy showed no degenerative alterations of stroma or cellular structures. Contradictory results were obtained by Benjamin et al., who studied 66 patients affected by breast cancer and treated with different doses of mitoxantrone.²⁸ The patients who received no previous immunosuppressant medications were subjected to a myocardial biopsy, which showed structural alterations. These were characterized by dilation of the sarcoplasmic reticulum or degenerative vacuolization of the myocytes to the point where myofibrils were lost. These findings are typical of anthracycline toxicity.²⁹ Moreover, no significant modification of left ventricular ejection fraction was noted either in patients previously treated with anthracycline or in those not treated. Nevertheless, the authors observed a trend towards reduced systolic function with increasing cumulative dose of mitoxantrone. This result was confirmed by Villani et al., who studied 25 patients affected by lung cancer previously treated with 5-fluorouracil.³⁰ These authors found a reduction of more than 15% in left ventricular ejection fraction after a mitoxantrone cumulative dose lower than 84 mg/m² by radionuclide angiographic examinations.

The present study leads to different conclusions. This population showed no alteration of the electrocardiographic parameters. No significant differences in left ventricular function between the two groups were found. In particular, patients treated with a mi-

toxantrone cumulative dose of 96 mg/m² showed no reduction of left ventricular ejection fraction or fractional shortening, or modifications on Doppler indices of left ventricular systolic and diastolic function during follow-up. These findings have several possible explanations. First, the authors studied only young patients who had not been exposed to previous cardiotoxic therapies or mediastinal radiation and were without pre-existing cardiac pathology. Second, they used low doses of mitoxantrone, with an intermittent infusion schedule, to obtain a marked and prolonged lymphopenia to reduce the abnormal immunoreaction probably responsible for multiple sclerosis clinical exacerbations. This pharmacologic schedule is different from those used in previous reports to obtain an antineoplastic effect.²⁸ The present data, according to previous studies,^{26,31} suggest that the risk of mitoxantrone cardiotoxic effects is low when it is given in patients with no previous cardiotoxic therapies or pre-existing heart disease and at a cumulative dose lower than 160 mg/m². However, the authors cannot exclude the possibility that, despite the absence of left ventricular ejection fraction reduction, a microscopic structural alteration of the myocardium, demonstrable only by serial biopsies, could be present.

In conclusion, mitoxantrone treatment in selected patients at a total dose of 96 mg/m² for 1 year shows no cardiac toxicity. Moreover, echocardiographic assessment of left ventricular function represents the method of choice for mitoxantrone treatment follow-up because of its safety, noninvasiveness, and accuracy.

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Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: a randomised multicentre study of active disease using MRI and clinical criteria

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Abstract

Objective—To evaluate the efficiency of mitoxantrone in multiple sclerosis.

Methods—Forty two patients with confirmed multiple sclerosis, selected as having a very active disease on clinical and MRI criteria were randomised to receive either mitoxantrone (20 mg intravenously (IV) monthly) and methylprednisolone (1 g iv monthly) or methylprednisolone alone over six months. In the steroid alone group five patients dropped out due to severe exacerbation.

Results—Blinded analysis of MRI data showed significantly more patients with no new enhancing lesions in the mitoxantrone group compared with the steroid alone group, (90% v 31%, $P < 0.001$). In the mitoxantrone group there was a month by month decrease almost to zero in the number of new enhancing lesions, and in the total number of enhancing lesions, whereas both remained high in the steroid alone group. The differences were significant for both indices at all months from 1–6. Unblinded clinical assessments showed a significant improvement in change in EDSS at months 2–6 in the mitoxantrone group, with a final mean improvement of more than one point (-1.1 v 0.3 ; $P < 0.001$). There was a significant reduction in the number of relapses (7 v 31; $P < 0.01$), and an increase in the number of patients free of exacerbation (14 v 7; $P < 0.05$).

Conclusion—In this selected group of patients with multiple sclerosis with very active disease, mitoxantrone combined with methylprednisolone was effective in improving both clinical and MRI indices of disease activity over a period of six months whereas methylprednisolone alone was not. Further double blinded long term studies are needed to properly evaluate the effect of mitoxantrone on progression in disability.

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Keywords: multiple sclerosis; magnetic resonance imaging; mitoxantrone; methylprednisolone

Immunological mechanisms have long been

thought important in the pathogenesis of multiple sclerosis, and several controlled trials of immunosuppressive therapy have been undertaken.¹⁻⁷ With the possible exception of interferon- β ,⁸⁻¹⁰ these trials have only shown a modest, if any, beneficial effect on the course of the disease.

Mitoxantrone is an anthracenedione antineoplastic agent that intercalates with DNA¹¹ and exerts a potent immunomodulating effect that suppresses humoral immunity,¹² reduces T cell numbers, abrogates helper activity, and enhances suppressor function.¹³ It is highly effective in suppressing the development of acute experimental allergic encephalomyelitis (EAE)^{14,15} and prevents or delays relapse in a chronic relapsing model of EAE.¹⁶ Studies by Gonsette and Demanty¹⁷ and later workers¹⁸⁻²² have suggested a possible therapeutic effect of mitoxantrone on multiple sclerosis. We have therefore evaluated the efficacy of mitoxantrone using both clinical and MRI criteria in a group of patients with multiple sclerosis selected as having a very active disease.

Methods

From October 1992 to October 1994, neurologists from five French University hospitals assigned 42 patients with very active multiple sclerosis to receive mitoxantrone (20 mg intravenously (iv)/month) and methylprednisolone (1 g iv/month) or methylprednisolone alone (1 g iv/month) by the trial randomisation code for a period of six months.

SELECTION OF PATIENTS

There was a two step selection. Patients were first selected clinically, then on MRI criteria. The clinical inclusion criteria were diagnosis of clinically definite multiple sclerosis (Poser criteria), age between 18 and 45 years, duration of disease less than 10 years. The clinical criteria for disease activity were either two relapses with sequelae within the previous 12 months or progression of two points on the EDSS scale during that time in those with secondary progressive disease. Relapsing-remitting course was defined as the occurrence of exacerbations followed by complete or partial remission, but without slow progression of disability between the relapses. Secondary progressive course was defined by the occurrence of a slow worsening of the disability lasting

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more than six months, with or without relapses, in patients having had, before the progression phase, a relapsing-remitting course.²³ Patients with relapsing disease had not had intensive steroid therapy for at least one month at the time of selection, or immunosuppressive agents within the past three months. Patients had to be ambulant (EDSS 6 or less). Patients with concomitant systemic disease, cardiac disease, and mental deficit were excluded. Women of child bearing age were required to have effective birth control.

During a baseline period of two months (month -2, month -1, month 0), three monthly gadolinium enhanced MRI scans were performed. During this baseline period, and throughout the trial, single iv injections of methylprednisolone (1 g after each monthly MRI) were given to all patients because it was considered appropriate to offer some active therapy in view of their highly aggressive course. Only patients developing at least one active MRI lesion during the baseline period were randomised. The allocation of the treatment at month 0 was done after inclusion by a central randomisation service by fax.

Eighty five patients fulfilled the clinical inclusion criteria. Forty three were then excluded, 36 who had no new lesions on MRI, three who had borderline abnormalities on echocardiography, three who had other clinical adverse events before entry, and one who had a severe relapse at entry and was too ill to participate. Accordingly, 42 patients were randomised into two groups: 21 to receive mitoxantrone combined with methylprednisolone, 21 to have methylprednisolone alone. Additional courses of steroid (methylprednisolone; 1 g/day iv for three days) were allowed for relapses.

MRI PROTOCOL

The MRI imaging protocol (used monthly at each neurological centre) was that proposed by the European Concerted Action guidelines.²⁴ Axial 5 mm thick slices were obtained through the brain with proton density and T2 weighted spin echo (SE) images before contrast, and a T1 weighted SE sequence after injection of gadolinium DTPA (0.1 mmol/kg).

ASSESSMENT AND FOLLOW UP

The MRI analysis was conducted at the NMR research unit, Institute of Neurology, London, by two observers who were totally blinded to the patients' clinical status, to randomisation, (treatment schedule), and had no contact at all with the patients throughout the study. The assessments of activity on the gadolinium enhanced and T2 weighted images were performed independently of one another. The size of new T2 lesions was classified as small if the maximum diameter was less than 5 mm, medium if the maximum diameter was 5-10 mm, and large where the maximum diameter was more than 10 mm.

Clinical assessments (including EDSS and recording of relapses) were carried out in each centre every month during the baseline period

and until completion of the trial. The relapses were documented by neurological examination, marked by the occurrence of symptom(s) of neurological dysfunction lasting more than 48 hours and preceded by stability or improvement for at least 30 days. These assessments were blind to MRI data but, for practical reasons, were not blind to treatment group.

Haematological and liver function tests and ECG were carried out monthly. Echocardiography was performed at entry and exit from the trial. The trial had full ethical approval.

MAJOR OUTCOME CRITERIA AND STATISTICAL ANALYSIS

The primary outcome criterion was the proportion of patients developing or not developing new enhanced lesions on serial gadolinium enhanced scans performed each month. The secondary criteria were the mean number of new enhanced lesions per month per patient, the number of new T2 lesions between month 0 and exit, and the monthly clinical outcome as assessed by EDSS and number of exacerbations.

Clinical and MRI differences between the two groups were tested for significance using non-parametric methods (Wilcoxon test), and differences between proportions with the χ^2 test. The P values were based on a two tailed test.

Results

There was no difference between the two patient groups in age, sex ratio, age at onset, duration of the disease, and total number of relapses since onset of multiple sclerosis (table 1). Six patients in the non-mitoxantrone group and four patients in the mitoxantrone group had secondary progressive multiple sclerosis whereas the remainder had relapsing-remitting disease. The EDSS at month -2 indicated moderate to severe disability in both groups (mean EDSS 4.7, and 4.4 in the non-mitoxantrone and mitoxantrone groups respectively), indicating relatively severe handicap with respect to disease duration. There were an average of 2.4 and 3.1 relapses within the 12 previous months in the non-mitoxantrone and mitoxantrone groups respectively.

Table 1 Characteristics of patients

Variable	MP (n = 21)	MP + MX (n = 21)
Age (y)	32.2 (8.1)	31.4 (8.3)
Sex ratio (M/F)	10/11	6/15
RRMS/SPMS ratio	15/6	17/4
Age at MS onset (y)	26.6 (6.5)	25.1 (7.0)
Duration of MS (y)	5.7 (4.0)	6.9 (3.6)
EDSS at month -2	4.7 (1.5)	4.4 (1.8)
No of exacerbations since MS onset	6.1 (3.7)	7.4 (4.5)
No of exacerbations in the 12 months preceding entry	2.4 (1.7)	3.1 (1.8)

Data are means (SD). There were no significant differences between the groups. MP = treated with methylprednisolone alone; MP + MX = treated with methylprednisolone + mitoxantrone; M/F = male/female; EDSS = expanded disability status scale; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

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Table 2 Description of the five patients who dropped out*

Time at drop out (months)	EDSS at month -2	EDSS at drop out	No of relapses from month -2	No of new enhancing lesions from month -2	No of scans with new enhancing lesions
4	5.5	6.5	4	35	6/7
4	4.5	5.0	3	14	7/7
4	6.0	8.5	3	98	7/7
3	6.0	7.5	2	51	6/6
5	4.5	8.0	3	99	7/7

*All five patients dropped out because of apparent lack of effectiveness and were in the methylprednisolone group. EDSS = expanded disability status scale.

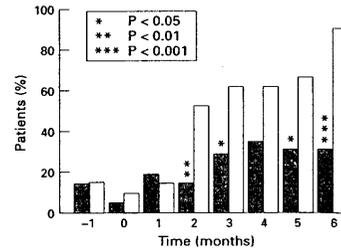
During the baseline period, 42 patients who satisfied both clinical and MRI criteria for randomisation had 21 relapses, giving an annual relapse rate of three, similar to their relapse rate during the preceding 12 months. By contrast, in the 36 patients who satisfied the clinical criteria for randomisation, but were excluded for lack of new MRI lesions, there were only four relapses during the baseline period, giving an annual rate of 0.7. This difference was significant ($P < 0.01$). During treatment, five patients dropped out at months 3, 4, or 5, because of pronounced deterioration. All were in the steroid only group, and had highly active disease both on clinical and MRI criteria (table 2). The patients were withdrawn to receive immunosuppressive treatment.

PRIMARY END POINT: PERCENTAGE OF PATIENTS WITHOUT NEW ENHANCING LESIONS

At entry (month 0), the percentage of patients without new enhancing lesions was 4.8% and 10% in the non-mitoxantrone and mitoxantrone groups respectively. During the treatment period, in the mitoxantrone group, starting from month 2 onward, this percentage increased and reached 90.5% by month six (figure). In the non-mitoxantrone group, there was a much smaller increase to 31.3%. This difference was highly significant. Differences at months 2, 3, and 5 were also significant, and in favour of the mitoxantrone group.

SECONDARY END POINTS

During the baseline period, the mean monthly



Percentage of patients without new active lesions (MRI) each month after monthly iv injection of 1 g methylprednisolone (grey bars) or 1 g methylprednisolone + 20 mg mitoxantrone (open bars). M-1 = one month before inclusion (M0); M1 to M6 = one to six month after inclusion. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

number of new enhancing MRI lesions varied between 4.6 and 9.1. During the treatment period, the mean monthly number of new enhancing lesions varied between 2.9 and 13.2 in the non-mitoxantrone group and between 0.1 and 2.6 in the mitoxantrone group. The number was less in the mitoxantrone group in all months, and the differences were significant from months 1 to 6 (table 3). The comparison of the monthly mean number of enhancing lesions (new lesions and persisting enhancing lesions) also showed significant differences in months 1 to 6 (table 3).

New and total (total = new and persisting enhancing lesions) contrast enhanced lesion frequency during the baseline period compared with the treatment period were significantly different in the mitoxantrone group whereas there were no statistical differences between these two periods in the non-mitoxantrone group (table 4).

We compared the T2 images at exit (month 6 for 36 patients, month 4 for four patients) with those at month 0. The mean number of new moderate, large, and total T2 lesions was significantly lower in the mitoxantrone group (table 5).

Table 3 MRI: number of new and total enhancing lesions

	Month†									
	-2	-1	0	1	2	3	4	5	6	6
Number of new enhancing lesions:										
MP										
n	—	20*	21	21	21	21	20‡	16‡	16‡	16‡
Mean (SD)	—	9.1 (17.9)	5.1 (5.7)	12.3 (28.8)	5.7 (7.5)	9.2 (25.8)	8.9 (16.7)	3.8 (5.3)	2.9 (3.2)	2.9 (3.2)
Median (range)	—	2.5 (0-78)	3 (0-23)	5 (0-135)	2 (0-26)	2 (0-120)	1 (0-65)	1 (0-17)	2 (0-11)	2 (0-11)
MP + MX										
n	—	20*	20*	21	21	21	21	21	21	21
Mean (SD)	—	6.8 (8.3)	4.6 (4.6)	1.9 (1.4)	2.6 (5.7)	1.1 (2.7)	0.9 (1.6)	0.6 (1.5)	0.1 (0.5)	0.1 (0.5)
Median (range)	—	3 (0-32)	3 (0-18)	2 (0-5)	0 (0-21)	0 (0-12)	0 (0-7)	0 (0-7)	0 (0-2)	0 (0-2)
P value (W)	—	NS	NS	< 0.05	< 0.05	< 0.05	< 0.05	< 0.01	< 0.001	< 0.001
Total number of enhancing lesions:‡										
MP										
n	20*	21	21	21	21	21	20‡	16‡	16‡	16‡
Mean (SD)	8.2 (8.6)	10.2 (18.6)	6.3 (6.7)	13.1 (28.6)	6.5 (7.8)	9.8 (25.7)	9.7 (17.3)	4.2 (5.7)	3.1 (3.2)	3.1 (3.2)
Median (range)	6 (0-32)	4 (0-81)	4 (0-24)	6 (0-135)	3 (0-26)	3 (0-120)	2 (0-68)	2 (0-19)	2 (0-11)	2 (0-11)
MP + MX										
n	20*	20*	21	21	21	21	21	21	21	21
Mean (SD)	7.1 (8.3)	9.5 (12.2)	5.7 (6.3)	3.3 (4.0)	3.6 (7.6)	2.5 (6.6)	2.3 (6.6)	1.9 (6.0)	1.4 (5.7)	1.4 (5.7)
Median (range)	5 (0-30)	3.5 (0-48)	3 (0-22)	2 (0-18)	1 (0-31)	0 (0-28)	0 (0-30)	0 (0-27)	0 (0-26)	0 (0-26)
P value (W)	NS	NS	NS	< 0.05	< 0.05	< 0.01	< 0.05	< 0.01	< 0.001	< 0.001

MP = Treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone. n = patient numbers; *One MRI was uninterpretable; †number of months before or after inclusion (M0); ‡patients dropped out because of severe deterioration (see table 2); §new + persisting lesions; W = Wilcoxon test.

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Table 4 Number of new and total contrast enhanced lesions during two months of pretreatment and six months of treatment

Variable	Baseline period (M1-M2)	Treatment period (M1-M6)	P value (W)
Number of new enhancing lesions per scan:			
MP			
Mean (SD)	7.0 (13.0)	7.5 (18.3)	NS
Median (range)	3 (0-78)	2 (0-135)	
MP + MX			
Mean (SD)	5.6 (6.7)	1.2 (2.9)	< 0.001
Median (range)	3 (0-32)	0 (0-21)	
Total number of enhancing lesions per scan:			
MP			
Mean (SD)	8.3 (12.5)	8.1 (18.4)	NS
Median (range)	5 (0-81)	3 (0-135)	
MP + MX			
Mean (SD)	7.5 (9.2)	2.5 (6.1)	< 0.001
Median (range)	4 (0-48)	0 (0-31)	

MP = Treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone; (M1-M2) = two month period before inclusion; (M1-M6) = six month period before inclusion; (M1-M6) = six month treatment period after inclusion; W = Wilcoxon test.

Table 5 Number of new lesions on the T2 weighted scans between inclusion (month 0) and end of study (month 6)

Variable	MP (n = 20*)	MP + MX (n = 20*)	P value (W)
New small lesions:			
Mean (SD)	1.7 (2.8)	0.6 (1.1)	NS
Median (range)	1 (0-11)	0 (0-4)	
New moderate lesions:			
Mean (SD)	2.3 (4.0)	0.5 (0.8)	< 0.05
Median (range)	1 (0-17)	0 (0-3)	
New large lesions:			
Mean (SD)	1.6 (3.2)	0.1 (0.2)	< 0.01
Median (range)	0.5 (0-14)	0 (0-1)	
Total new lesions:			
Mean (SD)	5.5 (9.0)	1.1 (1.4)	< 0.05
Median (range)	3 (0-38)	1 (0-5)	

One MRI was not interpretable; MP = treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone; W = Wilcoxon test; small: < 5 mm; moderate: 5 to 10 mm; large: > 10 mm.

EDSS

There were significant differences between the groups during the treatment period. Mean EDSS was better in the mitoxantrone group but this was significant only in month 4 (table 6). Change in EDSS with respect to month 0 was significantly better from months 2-6 (table 6). The mitoxantrone group improved in all months, with a final mean improvement of about one point compared with month 0. By contrast, the non-mitoxantrone group deteriorated from month 0 to month 4. Apparent improvement at months 5 and 6 was seen after the drop out of five patients due to severe deterioration. Furthermore, the distribution of the confirmed variation of one point EDSS (table

Table 6 EDSS during two months of pretreatment and six months of treatment

	Month†								
	-2	-1	0	1	2	3	4	5	6
EDSS:									
MP									
n	21	21	21	21	21	21	20‡	17‡	16‡
Mean (SD)	4.7 (1.5)	4.5 (2.0)	4.6 (1.7)	4.9 (2.1)	4.9 (1.8)	5.0 (1.7)	5.1 (1.8)	4.5 (2.1)	4.3 (2.1)
MP + MX									
n	21	21	21	12	21	21	21	21	21
Mean (SD)	4.4 (1.9)	4.5 (1.7)	4.5 (1.6)	4.2 (1.6)	4.1 (1.7)	3.9 (1.8)	3.6 (2.0)	3.4 (1.9)	3.4 (1.9)
P value (W)	NS	NS	NS	NS	NS	NS	< 0.05	NS	NS
Change in EDSS‡:									
MP									
n	—	—	—	21	21	21	20‡	17‡	16‡
Mean (SD)	—	—	—	0.2 (1.3)	0.3 (1.2)	0.3 (1.1)	0.6 (1.3)	0.1 (1.2)	-0.1 (1.1)
MP + MX									
n	—	—	—	21	21	21	21	21	21
Mean (SD)	—	—	—	-0.3 (0.7)	-0.4 (0.8)	-0.6 (0.8)	-0.9 (0.9)	-1.1 (1.0)	-1.1 (1.1)
P value (W)	—	—	—	NS	< 0.05	< 0.01	< 0.001	< 0.01	< 0.05

MP = Treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone; EDSS = Expanded disability status scale; n = patient numbers; *number of months before or after inclusion (m0); †changes in EDSS referred to M0 (inclusion); ‡five patients dropped out (see table 2); W = Wilcoxon test.

Table 7 Number and percentage of patients with one point confirmed variation* on EDSS between M0 (inclusion) and the end of the study

Variable	MP	MP + MX	P value (χ²)
Deterioration	6 (28.6)	1 (4.8)	< 0.01
Stable	12 (57.1)	8 (38.1)	
Improvement	3 (14.3)	12 (57.1)	

MP = Treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone; EDSS = expanded disability status scale; changes from 6.0 to 6.5 and from 6.5 to 7.0 on EDSS were considered equivalent to one point change. *The one point variation was measured for two months running, at the end of the study.

7) between month 0 and the end of the study was clearly different, showing that in the mitoxantrone group 12 out of 21 patients improved one point or more on the EDSS and only one deteriorated. By contrast, in the non-mitoxantrone group, six deteriorated and only three improved.

EXACERBATIONS (TABLE 6)

During the two month baseline period, the mitoxantrone and non-mitoxantrone groups had 12 and nine exacerbations respectively, giving calculated annual rates of 3.4 and 2.6, similar to the 12 preceding months. However, during the treatment period, there were fewer relapses in the mitoxantrone group (7 v 31 relapses). This effect was even more pronounced during the last four months of the treatment (1 v 18 relapses). Only five additional high dose steroid courses for relapses were given in the mitoxantrone group compared with 19 for the non-mitoxantrone group. During the treatment period, the number of patients free of exacerbations was 14 out of 21 in the mitoxantrone group and 7 out of 21 in the non-mitoxantrone group.

ADVERSE EVENTS

Several adverse events were recorded, more in the mitoxantrone group than in the non-mitoxantrone group. There was no evidence of serious side effects. In particular, no cardiotoxicity was detected. Six patients in the non-mitoxantrone group and 18 in the mitoxantrone group had at least one adverse event (table 9). Alopecia was only minor and transient for seven patients. Eight out of 15 women developed

Table 8 Relapses and patients free of exacerbations during two months of pretreatment and six months of treatment

Variable	MP	MP + MX	P value (χ^2 or W)
Relapses:			
Baseline period:			
Number of relapses	9	12	NS
Annual rate/patient	2.6	3.4	
Additional steroid courses (iv high doses)	5	6	
During treatment period:			
M0-M6			
Number of relapses	31	7	< 0.01
Annual rate/patient	3.0	0.7	
Additional steroid courses (iv high doses)	19	5	
M0-M2			
Number of relapses	13	6	
Annual rate/patient	3.7	1.7	
M3-M6			
Number of relapses	18	1	
Annual rate/patient	2.6	0.1	
Number of patients free of exacerbations:			
Baseline period:			
Number of patients free of exacerbations	13	10	NS
During treatment period:			
M0-M6			
Number of patients free of exacerbations	7	14	< 0.05
M0-M2			
Number of patients free of exacerbations	11	15	
M3-M6			
Number of patients free of exacerbations	9	20	

MP = treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone; M0 = inclusion; M2 = two months after inclusion etc. The separation between M0-M2 and M3-M6 illustrate the difference between the first and the second part of the trial; W = Wilcoxon test.

Table 9 Number of patients with adverse events

Variable	MP	MP + MX
Amenorrhoea	—	8
Alopecia	—	7
Nausea, vomiting	—	6
Other digestive events	1	6
Other cutaneous events	2	5
Asthenia	—	5
Upper tractus infection	2	5
Urinary infection	1	4
Other neurological events	—	3
Tachycardia, palpitation	1	1
Hepatitis	1	—
Headache	1	—
Menstrorrhagia	—	1
Others events	1	4
Haematological abnormality at the initiation of the next treatment course:		
Leucopenia (< 3000/mm ³)	—	2
Anaemia	1	4

MP = Treated by methylprednisolone alone; MP + MX = treated by methylprednisolone + mitoxantrone.

amenorrhoea beginning at month 2 (two patients), at month 3 (three patients), at month four (one patient), at month 5 (one patient), and at month 6 (one patient) after starting mitoxantrone. Amenorrhoea was transient for seven women and persistent for one woman aged 44. All patients in the mitoxantrone group had an expected pronounced leucopenia about two weeks after injection, which disappeared within a few days. At the next monthly injection, leukopenia was minor (World Health Organisation (WHO) grade 2) for two patients, and anemia was also minor (grade 1 WHO) for four patients without the need for dose adjustment. Nine patients had concomitant treatment for nausea before receiving mitoxantrone (granisetron (3 mg iv)). No drop outs occurred in the mitoxantrone group. All the patients receiving mitoxantrone had full dose injections as scheduled. There were no serious infections, no patient developed moderate or severe alopecia, or moderate or severe gastrointestinal events.

Discussion and conclusions

In recent years, there has been increasing use

of frequent serial MRI to obtain an initial assessment of the efficacy of new therapies on disease activity in multiple sclerosis.²⁴⁻²⁸ Such an approach in early relapsing-remitting and secondary progressive multiple sclerosis can show treatment effects within a matter of months in only a few patients, because serial MRI shows much more disease activity than is clinically apparent. We therefore used monthly gadolinium enhanced MRI as the primary outcome measure in this short term study of the efficacy of mitoxantrone. The study showed a pronounced and highly significant reduction in the frequency of new enhancing lesions in the group treated with mitoxantrone and methylprednisolone compared with the group treated with methylprednisolone alone. It is notable that we saw a much higher rate of enhancing lesions than T2 lesions. This can partly be related to the fact that there was a greater frequency of sampling (monthly v six monthly), but even when gadolinium enhanced and T2 weighted scans are analysed at the same (monthly) intervals, it has been shown that there is an appreciably higher level of activity detecting using enhancement.²⁹ The explanations for this increase are many and include re-enhancement of old lesions, and a generally greater conspicuity of small areas of new activity on enhanced images.

Clinical trial methodology normally demands double blinding. In the present study, although the allocation of treatment was performed using an unbiased randomisation service, neither the patients nor the clinical investigators were blinded during the study. Blinding of patients was not possible in this trial, as obvious side effects of mitoxantrone were experienced in almost all cases. Blinding of the physician was made difficult by the fall in white cell count that always accompanies mitoxantrone treatment. Blind clinical observers might have been appointed, but this could not be done for economic reasons. The clinical efficacy suggested in this study must therefore be regarded with caution as it was acquired unblinded.

Looking at the non-mitoxantrone group gives us the opportunity to assess the effect of methylprednisolone alone. These patients had regular monthly methylprednisolone injections in addition to a three day course of methylprednisolone for relapses as needed. The regular monthly treatment was done because we thought that some treatment should be offered to patients with such aggressive disease. The efficacy of methylprednisolone alone was clearly poor. The non-mitoxantrone group required 19 additional steroid courses for severe exacerbations. Despite the regular, but intermittent methylprednisolone treatment, the number of patients with new enhancing MRI lesions decreased only slightly and non-significantly. This slight improvement may be a natural history phenomenon (regression to the mean) and not a steroid effect. The effect of a three day course of iv methylprednisolone on enhancing lesions probably lasts for less than one week.³⁰ This marginal improvement on

MRI was, moreover, not associated with clinical benefit as five patients in this group dropped out of the trial because of pronounced deterioration, the relapse rate did not decrease, and the EDSS did not improve.

Open pilot studies of mitoxantrone treatment have suggested some therapeutic effect.¹⁷⁻²⁰ A recent controlled study²¹ showed a reduction in relapses, but no significant difference in the EDSS scale or in the development of active MRI lesions. This study was of 13 patients, and the patient group had less active disease (a baseline relapse rate of about half) than in the present study. MRI criteria were not used in patient selection, and evaluations were carried out less often. The inclusion criteria used in the present study were specifically aimed at selecting informative patients, and the number studied was sufficient to show meaningful changes. It is likely that this accounts for the fact that we have found a therapeutic effect on MRI in our study.

Mitoxantrone has been widely used as an anticancer drug for more than 10 years.^{31,32} The dose used in oncology is much higher than in multiple sclerosis studies and the main risk, that of cardiotoxicity, seemed to be low at the doses used in the present study (about 70 mg/m²). No cases were seen in the present study despite very careful cardiac monitoring, confirming the results of De Castro *et al.*²² and patients were excluded if there were clinical, ECG, or echocardiographic features of cardiac abnormalities. However, although the dose used in this study seemed safe, we cannot dismiss the possibility that subclinical, permanent, and minor cardiac injury induced by the drug could later become clinically symptomatic as patients age and develop the common forms of heart disease. There are three reasons why the results of the present trial do not allow us to draw conclusions as to the long term clinical efficacy of mitoxantrone on the course of multiple sclerosis:

- (1) The relation between short term gadolinium enhancement and long term disability is uncertain; preliminary experience suggests that a relationship exists³³ but confirmation from larger studies is needed.
- (2) The apparent short term clinical benefits we saw were unblinded observations and are therefore not wholly reliable.
- (3) We cannot exclude the possibility that part of the benefit we saw in the mitoxantrone group came from the addition of methylprednisolone to the treatment regime.

None the less, it was clear in this selective group of patients with multiple sclerosis with very active disease that the combination of mitoxantrone and methylprednisolone greatly improved objective and blinded MRI indices of disease activity over six months, whereas methylprednisolone alone did not. The strong and rapid reduction in the inflammatory process suggests a potential role for mitoxantrone as rescue therapy or as an induction for other long term disease modifying therapies in very active cases of multiple sclerosis. Its effect on the long term clinical course can only be determined by longer term trials.

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Mitoxantrone in Progressive Multiple Sclerosis: A Placebo-Controlled, Randomized, Observer-Blind Phase III Trial: Clinical Results and Three-Year Follow-up

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OBJECTIVE: To determine the efficacy of mitoxantrone (MX) in the treatment of relapsing progressive or secondary progressive multiple sclerosis (MS).

BACKGROUND: MX is a cytotoxic agent of the anthra-cenedione family. Its main mechanisms are intercalation with DNA and inhibition of topoisomerase II. It is immunosuppressive by reducing the number of B cells, inhibiting T helper cell function and augmenting T cell suppressor activity. MX is effective in experimental allergic encephalomyelitis and showed a beneficial effect in several Phase II clinical trials in MS.

DESIGN/METHODS: In this European multicenter phase III trial 194 patients with relapsing-progressive or secondary-progressive MS were randomized to 12 mg/m² mitoxantrone (MX12), 5 mg/m² mitoxantrone (MX5) or placebo. The medication was given intravenously three monthly for two years. A neurological examination was done every three months by a blinded observer to determine EDSS and Ambulation Index (AI). Relapses were treated for five days with intravenous methylprednisolone (500 mg/day). Main efficacy criteria were Δ-EDSS, Δ-AI, number of treated relapses and time to 1st treated relapse. Secondary clinical efficacy parameters were the proportion of patients with confirmed EDSS progression, time to confirmed EDSS progression, number of all relapses, time to first relapse, proportion of patients with no relapses and number of hospitalizations. Primary efficacy parameters were first tested by a multivariate generalized Wilcoxon-Mann-Whitney (WMW) test and then according to the principle of a priori ordered hypotheses by WMW tests or log rank test.

RESULTS: 188 patients were evaluable in the intent-to-treat analyses. There were no significant differences in the baseline parameters between the three groups. Mean age was 41 years and mean duration of MS 10 years. Patients had 1.3 relapses in the year before study entry and the deterioration in the EDSS was 1.57 in the previous 18 months. Baseline EDSS values were 4.5, 4.6 and 4.7 in MX12, MX 5 and PLC respectively. The multivariate test was statistically significant

favouring MX12 ($p < 0.001$). We could show statistically significant results in each primary endpoint. EDSS was decreased by 0.12 in MX12, 0.23 in MX5 and increased in the placebo group by 0.23 ($p < 0.038$). AI was increased with 0.2, 0.4 and 0.8 for MX 12, MX 5 and PLC, respectively ($p < 0.040$). The mean number of treated relapses were 0.4 for MX12, 0.7 for MX5 and 1.2 for PLC ($p < 0.0002$). There was a statistically significant difference in the time to first treated relapse ($p < 0.0004$). MX was also effective on all secondary endpoints. Three year follow-up data will be presented. Adverse events reported more frequently in the MX groups were nausea, alopecia, urinary tract infections, menstrual disorders, amenorrhoea, transient leucopenia and increase of γ -glutamyltransferase.

CONCLUSION: In this study mitoxantrone shows a beneficial effect in the treatment of patients with progressive multiple sclerosis. Generally, patients treated with MX12 showed a more favourable response. Adverse events were in line with previous experiences.

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**Mitoxantrone in Progressive Multiple Sclerosis (MS):
A Placebo-Controlled, Randomized, Observer-Blind
European Phase III Multicenter Study - Clinical Results**

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Background: Mitoxantrone (MX) is a potent anthracenedione immunosuppressor, very effective in experimental allergic encephalomyelitis and with a beneficial effect in several Phase II clinical trials in MS.

Material and Methods: 194 patients with progressive MS were randomized to 12mg/m² MX, 5mg/m² MX or placebo (methylene-blue). The medication was given intravenously every three months (m) for two years.

Results: 188 patients were evaluable. There were no significant differences for baseline variables in the three groups. After 24 months the following results were obtained:

	12mg/m ²	5mg/m ²	Placebo
-EDSS	-0.130.90 *	-0.231.10 *	0.231.01
-Ambulation- Index	0.301.24 *	0.411.40 *	0.771.26
Annual Relapse Rate	0.21 **	0.36 *	0.60
Time to 1st relapse (m)	>24 **	>24 **	15
Confirmed Treatment failure	7%*	9%	19%

* p<0.05; **p<0.001 (comparison to placebo)

MX was well tolerated. No major toxicity, including cardiotoxicity, was noted.

Conclusion: These data demonstrate a significant beneficial effect of MX on relapse rate and disability progression. MRI data confirm the beneficial effect of MX on disease activity.

Hartung HP, Gonsette R, and the MIMS-Study Group. Supplement 1, 1999

Mitoxantrone in Progressive Multiple Sclerosis (MS): Clinical Results and Three-Year Follow-Up of the MIMS Trial.

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A³ doubleblind phase III trial was performed to demonstrate the safety and efficacy of mitoxantrone (MX) in multiple sclerosis (MS). 194 patients with relapsing-progressive or secondary-progressive MS in an active stage of disease (EDSS deterioration ≥ 1 in the year prior to entry) were randomized to either 12mg/m² MX (MX12), or 5mg/m² MX (MX5) or placebo (P). Treatment was given intravenously every three months over two years. Patient and rating physician were blinded. After three years a follow-up examination was performed.

Compared to P, MX12 significantly slowed the disease progression over the two-years study period as shown by its effect on change in EDSS (-0.13 vs 0.23; p=0.019), change in ambulation index (0.30 vs 0.77; p=0.031) and proportion of patients with three months confirmed EDSS-deterioration (8% vs 22%; p=0.036). There was also an effect on mean number of treated relapses (0.4 vs 1.2; p=0.0002) and time to 1st relapse. A clear dose-response effect was evident with maximum benefit gained at MX12. Follow-up data showed, that these beneficial effects were sustained. Adverse events (AE) reported more frequently in MX were nausea, alopecia, urinary tract infections and menstrual disorders. Three years follow-up data showed that AE were reversible in most cases. There was no evidence of serious cardiotoxicity.

Based on this study, MX12 given every three months appears to be an effective and well tolerated treatment for patients with active secondary progressive or relapsing-progressive MS.

Krapf H, Morrissey SP, Zenker O, Gonsette R, et al. 1998

P3024

Mitoxantrone in Progressive Multiple Sclerosis (MS): A Placebo-Controlled, Randomized, Observer-Blind European Phase III Multicenter Study - MRI Results

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Background: In a recently finished large Phase III trial, mitoxantrone (MX) has shown a beneficial impact on clinical disease progression and relapse rate in relapsing-progressive MS. In a subgroup of these patients MRI was carried out as a surrogate marker of disease activity.

Material and Methods: 110 patients were treated intravenously with 12 mg/m² MX, 5 mg/m² MX or placebo (methylene-blue) every 3 months. MRI served as a secondary endpoint measure: T2-weighted (w) axial images (conventional double spin-echo, TR/TE 2500/90/45 ms) and T1-w images (TR/TE 600/20 ms) after Gd-DTPA injection (0.1 mmol/kg) were acquired at months (M) 0, 12 and 24.

Results: Statistically significant changes were found for: 1. *T2-w MRI:* There was almost no progression detectable in both treatment groups in regard to the lesion load, whereas in the placebo group a continuous increase was found at M 12 and M 24 ($p < 0.05$). 2. *Gd-enhanced MRI:* There was a significant decrease of enhancing lesions in one treatment group as compared to the placebo-group ($p < 0.05$).

Conclusion: These MRI results clearly corroborate the beneficial impact of MX on the clinical outcome in progressive MS patients demonstrated in this trial. The clinical results are subject of a separate presentation.

Millefiorini E, Gasperini C, Pozzilli C, D'Andrea F, et al. 1997

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Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome

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Abstract We designed a randomized, placebo-controlled, multicentre trial involving 51 relapsing-remitting multiple sclerosis patients to determine the clinical efficacy of mitoxantrone treatment over 2 years. Patients were allocated either to the mitoxantrone group (27 patients receiving IV infusion of mitoxantrone every month for 1 year at the dosage of 8 mg/m²) or to the placebo group (24 patients, receiving IV infusion of saline every month for 1 year) using a centralized randomization system. Disability at entry and at 12–24 months was evaluated by four blinded neurologists trained in the application of the Kurtzke Expanded Disability Scale (EDSS). In addition, the number and clinical characteristics of the exacerbations over the 24 months were recorded by the local investigators. MRI, at 0, 12 and 24 months, was performed with a 0.2 T permanent unit. MRI data were analysed by two blinded neuroradiologists. All patients underwent a clinical evaluation. A statistically significant difference in the mean number of exacerbations was observed between the

mitoxantrone group and placebo group both during the 1st and the 2nd year. Although there was no statistically significant benefit in terms of mean EDSS progression over 2 years, the proportion of patients with confirmed progression of the disease, as measured by a one point increase on the EDSS scale, was significantly reduced at the 2nd year evaluation in the mitoxantrone group. Forty-two (23 mitoxantrone, 19 placebo) patients underwent all MRI examinations during the 24-month period. We observed a trend towards a reduction in the number of new lesions on T2-weighted images in the mitoxantrone group. Our study suggests that mitoxantrone might be effective in reducing disease activity, both by decreasing the mean number of exacerbations and by slowing the clinical progression sustained by most patients after 1 year from the end of treatment.

Key words Multiple sclerosis · Mitoxantrone · Magnetic resonance imaging

Introduction

Multiple sclerosis (MS) is an autoimmune disease associated with heightened immune activity against central nervous system antigens [9]. Several attempts to alter the course of MS have largely focused on strategies to down-

regulate the immune response by using a variety of immunosuppressive agents such as cyclophosphamide, cyclosporine and azathioprine [28, 30, 34]. High doses of these drugs may have a modest effect on the exacerbation rate and disease progression, as well as unacceptable side-effects. An alternative therapy for patients with MS that has similar or greater efficacy but is less toxic would there-

fore be desirable. One recent approach has been the use of mitoxantrone (MTX) as a therapeutic agent. MTX is an antineoplastic agent that intercalates with deoxyribonucleic acid and is a potent inhibitor of both deoxyribonucleic acid and ribonucleic acid synthesis [6, 19]. In vivo and in vitro studies indicate that MTX is an immunomodulator that reduces T cell numbers, suppresses humoral immunity and causes in vivo inhibition of T-helper cell, but not T-suppressor cell activity [7].

Preliminary open-label trials to assess the potential efficacy of MTX treatment, performed only in patients with progressive MS, suggested that MTX does not completely suppress clinical and magnetic resonance imaging (MRI) evidence of disease progression [8, 13, 21, 22]. Recent data, however, indicate that therapies in MS are more effective if used early, when there is less neurological damage and when the demyelinating process is just beginning [24].

On the basis of these assumptions, we designed a randomized, placebo-controlled, multicentre trial using relapsing-remitting multiple sclerosis (RRMS) patients to determine the clinical efficacy and toxicity of MTX over 2 years at the dosage of 8 mg/m² administered IV once a month for 12 months.

Patients and methods

This study was approved by internal review boards and by the National Health Service as a 2-year randomized, placebo-controlled multicentre trial. It was conducted at eight Italian centres: Bari, Catanzaro, Chieti, Naples (2 centres), Rome, Siena and L'Aquila, the last also being the Coordinating Centre.

Patient enrolment and pre-trial observation

Patients fulfilling the following inclusion criteria were enrolled in the study: age between 18 and 45 years, clinically definite or laboratory-supported RRMS [25], disease duration from 1 to 10 years, disability from 2 to 5 on the Kurtzke Expanded Disability Status Scale (EDSS) [16], with at least two exacerbations in the previous 2 years.

We excluded patients who were HIV-positive, with previous cardiovascular disease, with left ventricular ejection fraction of less than 50% as determined by echocardiography, subjects presenting renal, liver and/or respiratory dysfunction, diabetes, malignancy, psychiatric illness, pregnancy and women not practising contraception, as well as patients who had taken steroids during the 3 months before entry or previous immunosuppressant medications. Lastly, patients incapable of fulfilling the requirements of the study or signing the informed consent were also excluded.

When a patient became eligible, the investigators notified the relevant centre which validated the eligibility of the patient and assigned a randomization code number. The subject was then randomly assigned to a recipient group that received either MTX or a placebo. Patients were randomized to MTX or placebo using a scheme stratified on age, sex and EDSS which resulted in eight different age/sex/EDSS strata. According to the study protocol, within each stratum the allocation of patients to treatment or placebo was balanced by using a block design of size eight.

Efficacy end-points

Treatment efficacy was assessed by comparing the following variables between the placebo and MTX group.

Primary end-point

1. Proportion of patients with confirmed progression as measured by an increase of at least one point on the EDSS scale.

Secondary end-points

1. Annual mean number of exacerbations and proportion of exacerbation-free patients.
2. Change in mean EDSS from baseline to end-point.
3. Mean number of new or enlarged lesions on T2-weighted MRI performed at baseline and 12 and 24 months after the beginning of the study.

Patient evaluation and drug medication

Four neurologists were selected before the beginning of the study as EDSS physicians and each of them was assigned to two centres to evaluate the same patients. EDSS physicians were trained in the application of the EDSS during a joint session which included repeated rating of patients with MS with varying levels of disability. In order to measure the degree of interobserver (intercentre) discrepancies on EDSS evaluation, 16 definite, stable MS patients with an EDSS score ranging from 0 to 7 were randomly selected. Each patient was examined, at the same visit and after 1 week, independently by each of the four neurologists involved in the study. *K* statistics were used to define six levels of agreement (poor for *K* = 0, slight for *K* = 0–0.20, fair for *K* = 0.21–0.40, moderate for *K* = 0.41–0.60, substantial for *K* = 0.61–0.80, almost perfect for *K* = 0.81–1) [17]. An "almost perfect" (mean *K* value = 0.83 for patients in the 1.0–5.0 EDSS range) and a "moderate" (mean *K* value = 0.53 for patients in the 5.0–7.0 EDSS range) inter-observer agreement within one step of the EDSS scale were obtained. In order to maintain blindness, the interaction of the EDSS physicians with the patient was strictly restricted to the neurological examination. The neurologist was not allowed to talk with the patient about adverse events, or any other issue which could potentially disclose the patient's treatment. Neurological examination was rated using the EDSS prior to starting therapy and 12 and 24 months later.

Monitoring and recording of exacerbations, concomitant therapy or other medical events were documented throughout the study by a treating physician selected in each centre before the beginning of the study. The treating physician, not blinded to study treatment, was responsible for the subject's overall medical care, including physical examinations, evaluation of the patient's subjective findings, prescribing and monitoring the study medication, and evaluating and managing adverse events and exacerbations. Exacerbation was defined as the appearance of a new symptom or worsening of an old symptom, attributable to MS, accompanied by a documented new neurological abnormality, lasting more than 48 h and preceded by stability or improvement for at least 30 days.

Patients assigned to immunosuppressive treatment received a 30-min infusion of MTX intravenously (8 mg/m²) every month for 1 year; the intravenous bag and tubing were black to ensure patient blinding. Placebo group patients received a solution containing the vehicle alone.

Other drugs were allowed during the trial such as cholinergic and spasmolytic drugs or short courses of steroids (1 g/day methylprednisolone IV for 6 days) for exacerbations.

Safety evaluation

The safety of the treatment was assessed on the basis of adverse events volunteered by the patient either spontaneously or on questioning and monitoring of the main laboratory parameters.

Blood and urine samples were taken and ECG carried out upon entry to the trial and at each monthly visit. Moreover, to assess the potential cardiac toxicity of MTX, each patient had an M-mode and two-dimensional echocardiographic study with spectral and colour Doppler flow analysis, which was performed at enrolment and 6 and 12 months later. The images were recorded on super-VHS videotape and analysed according to the American Society of Echocardiography criteria [10] by evaluating left ventricular systolic function parameters, such as fractional shortening and ejection fraction.

MRI assessment

MRI studies, performed at 0, 12 and 24 months, were obtained with different imagers using T2 spin-echo sequences in the axial plane. Slices with 7.5 mm thickness without gaps between sections were obtained for all the studies. In order to obtain comparable studies during the follow-up, a midline sagittal scout slice was always performed at the beginning of the study. We therefore oriented axial sections on the same horizontal plane along a line passing through the base of the frontal lobe and the caudal portion of the quadrigeminal plate.

In addition, in a subgroup of patients (13 MTX and 12 placebo) a serial enhanced MRI study was performed at 0, 2, 4, 6 and 12 months from the beginning of the study [1].

Image evaluation

MRI data were analysed by two blinded neuroradiologists and questionable lesions were reviewed by a third blinded senior neuroradiologist acting as supervisor. Prior to the study, the neuroradiologists were trained to minimize inter- and intra-observer variability. Both the inter- and intra-rater variability during serial examinations was less than 5%.

Demyelinating areas seen on T2-weighted images were detected at the baseline MRI study. Follow-up T2-weighted images were obtained 12 and 24 months after the beginning of the study, and were analysed for new disease activity as revealed by the presence of new and enlarging lesions. New lesions were defined as lesions that were not present on T2-weighted MRI performed 1 year previously. An enlarging lesion was defined as a lesion with a change exceeding more than 33% (1/3) when compared with the MRI performed 1 year before.

Statistical analysis

For determination of sample size we assumed the detection of an EDSS increase of 1 point in 25% of the MTX-treated group compared with 50% of the placebo-treated group at the time of scheduled efficacy analysis. According to an alpha error of 0.05 and beta error of 0.20, the number of patients required to achieve statistical significance was 65 per treatment arm. An intention to treat analysis was used to analyse all clinical measures. Unfortunately, difficulties in patient recruitment did not allow us to achieve the required number.

Clinical and MRI differences between the two groups were tested for statistical significance by using non-parametric methods (Mann-Whitney test, Wilcoxon rank test and two-sided *P* values). The chi square test was used for categorical data, i.e. differences between proportions.

Results

Clinical outcome

The baseline demographic and clinical characteristics of 51 patients randomly assigned to the two groups are shown in Table 1. The groups were well balanced for age, disease duration, neurological disability and the number of exacerbations in the previous 2 years, while the incomplete recruitment generated an imbalance in term of sex. In the placebo group 9 patients had a baseline EDSS score of 2 or 3, and 15 patients of 4 or 5. In the MTX group, there were 7 patients with 2 or 3, and 20 patients with 4 or 5 as the baseline EDSS score.

As regards the proportion of patients with confirmed progression of the disease as measured by a one point increase on the EDSS scale, we found a negative trend in the MTX group (2/27) compared with the placebo group (6/24) in the 1st year, which became significant (*P* = 0.01) in the 2nd year (0/27 in the MTX group versus 6/24 in the placebo group) (Table 2). During the total period of the study (0–2 years) 9 of 24 (37%) patients in the placebo group progressed on the EDSS scale by at least one point (Table 2). The mean EDSS score of these 9 patients changed from 3.8, SD 0.8 at baseline to 5.2, SD 1.4 at the end of the 2nd year. Four of them reached an EDSS score ≥ 6. In

Table 1 Baseline clinical characteristics of patients (MTX mitoxantrone, EDSS Expanded Disability Status Scale)

	MTX	Placebo
No. of patients (male/female)	27 (10/17)	24 (6/18)
Age (years) (mean, SD)	30.9, 6.0	28.7, 6.5
Age at onset (years) (mean, SD)	23.7, 5.6	24.3, 5.3
Disease duration (years) (mean, SD)	5.7, 3	5, 3
No of exacerbations in previous 2 years (mean, SD)	2.8, 1.2	2.8, 1.1
EDSS score	3.6, 0.9 range: 2–5 median: 3.5	3.5, 1.2 range: 2–5 median: 3.5

Table 2 Confirmed progression of disability by at least one point on the EDSS (CI confidence interval)

	MTX (n = 27)	Placebo (n = 24)	<i>P</i> value	95% CI of the differences
1st year	2/27 (7%)	6/24 (25%)	0.08	18 (0–38)
2nd year	0/27 (0%)	6/24 (25%)	0.01	25 (7–43)
Total period (0–2 years)	2/27 (7%)	9/24 (37%)	0.02	30 (8–52)

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MTX group only 2 of 27 (7%) patients progressed on the EDSS scale by at least one point, the first from 5.0 to 6.0 and the second from 3.5 to 5.5.

Although a significant worsening in EDSS score was observed in the placebo group between the baseline and the 2nd year evaluation (placebo: 3.5, SD 1.2 vs 4.2, SD 1.6, $P = 0.007$; MTX: 3.6, SD 0.9, 3.5, SD 1.4, $P = NS$), there were no significant differences between the two groups in the mean EDSS change after either the 1st or 2nd year of the study. A significant decrease in the mean annual number of exacerbations was observed in the MTX group compared with the placebo group both during the 1st year ($P = 0.001$) and the 2nd year ($P = 0.005$) (Table 2). Furthermore, we found a significant difference in the number of exacerbation-free patients: 6 of 24 (25%) in the placebo group versus 19 of 27 (70%) in the treated group ($P = 0.003$) in the 1st year and 10 of 24 (42%) in the placebo group versus 21 of 27 (78%) in the treated group ($P = 0.01$) in the 2nd year (Table 3).

MRI outcome

Nine of the 51 patients did not complete the MRI study for the following reasons: 2 MTX and 4 placebo patients felt the drug was not working, 1 MTX patient did not have good compliance with his neuroradiological centre, 1 MTX and 1 placebo patients withdrew for non-medical reasons. We did not find significant differences in terms of demographics and clinical characteristics either at baseline or during the study between the 9 patients who did not complete the MRI study and those who did.

The mean number of new and enlarging lesions on T2-weighted images at 12 and 24 months of the patients in both treatment groups who completed the MRI study are shown in Table 4. No significant difference in the development of new and enlarging lesions was observed between the two groups either at 12 or 24 months after the beginning of the study. However, a reduction of 31% and 46% of new lesions respectively at 12 and 24 months was observed in the MTX group when compared with the placebo group.

Table 3 Effect of MTX on exacerbations: summary of 1st and 2nd year data

	MTX (n = 27)	Placebo (n = 24)	P value	95% CI of the differences
<i>1st year data</i>				
Number of exacerbations (mean, SD)	0.52, 1.1	1.54, 1.3	0.001	1.02 (0.36–1.68)
Exacerbation-free patients	19/27 (70%)	6/24 (25%)	0.003	45% (21–69)
<i>2nd year data</i>				
Number of exacerbations (mean, SD)	0.37, 1.0	1.1, 1.1	0.005	0.73 (0.18–1.28)
Exacerbation-free patients	21/27 (78%)	10/24 (42%)	0.01	36% (11–63)
<i>Total period (0–2 years)</i>				
Number of exacerbations (mean, SD)	0.89, 2.1	2.62, 1.9	0.0002	1.73 (0.62–2.84)
Exacerbation-free patients	17/27 (63%)	5/24 (21%)	0.006	42% (15–65)

Table 4 Mean and median number of new and enlarging lesions on MRI

	MTX (n = 23)	Placebo (n = 19)	P value	95% CI of the differences
<i>1st year</i>				
New lesions (SD)	2.5, 2.3	3.6, 4.1	0.6	-1.1 (-3.2 to 1)
Median	2*	2		
Enlarging lesions (SD)	1.8, 2.1	1.8, 2.7	0.8	0 (-1.5 to 1.5)
Median	1	1		
<i>2nd year</i>				
New lesions (SD)	1.8, 3.0	3.3, 4.1	0.07	-1.5 (-3.8 to 0.8)
Median	1	2		
Enlarging lesions	2.8, 3.8	2.6, 2.5	0.8	0.2 (-1.8 to 2.0)
Median	2	1		
<i>Total period (0–2 years)</i>				
New lesions (SD)	3.5, 3.4	7.3, 8.0	0.05	-3.8 (-7.7 to 0.1)
Median	2	5		
Enlarging lesions (SD)	4.3, 4.7	4.3, 4.7	1	0 (-1.5 to 1.5)
Median	3	3		

Table 5 Number of patients with adverse reactions. Five women developed transient amenorrhoea that was rated severe. Mild: adverse reactions did not require medication for control. Moderate: adverse reactions required medication for control (*URI* upper respiratory tract infection, *UTI* urinary tract infection)

	Total	Mild	Moderate	Severe
Nausea/vomiting	9 (18%)	7 (14%)	2 (4%)	0 (0%)
URI	2 (4%)	1 (2%)	1 (2%)	0 (0%)
UTI	3 (6%)	1 (2%)	2 (4%)	0 (0%)
Headache	3 (6%)	3 (6%)	0 (0%)	0 (0%)
Diarrhoea	1 (2%)	0 (0%)	1 (2%)	0 (0%)

Adverse effects

In general, MTX was well tolerated (Table 5). The most common side effect was nausea, which was generally mild and easily controlled with antiemetics. One patient had diarrhoea, vomiting and a slight fever. There were no serious infections; three patients experienced mild urinary tract infection and two patients upper respiratory tract infection, all of which resolved with antibiotic therapy. No patient developed moderate or severe alopecia or haematological adverse reactions. Five women developed transient secondary amenorrhoea 4 months (2 patients), 6 months (1 patient) and 8 months (2 patients) after starting MTX, though this resolved rapidly with the cessation of therapy. The electrocardiograms performed at entry were normal in all but two patients, who had a left bundle branch block. The subsequent electrocardiograms, performed during the study, showed no modification of wave morphology or appearance of arrhythmia in either the patients whose examination was normal at entry or in those with left bundle branch block. Moreover, no differences were observed in left ventricular systolic function measurements, such as fractional shortening and ejection fraction.

Discussion

The primary purpose of this trial was to determine whether monthly therapy with MTX at a dose of 8 mg/m² per month for 1 year could alter disease progression in RRMS patients. The results of this study indicate that MTX is well tolerated and has a beneficial effect on the short-term course of RRMS. We found MTX to have a significant effect on most primary and secondary efficacy end-points, including exacerbation rate, proportion of exacerbation-free patients and proportion of patients with a confirmed clinical progression. The reason why no statistically significant benefit could be detected in terms of mean EDSS progression over 2 years may be the limited number of patients included in this trial, which was lower than that required by statistical assumptions.

Our data showed a 66% reduction in the annual number of exacerbations in the MTX-treated group at the end of the treatment period (1st year) and at the end of the follow-up period (2nd year). In addition, the proportion of patients with a confirmed progression of the disease as measured by a one point increase on the EDSS scale, was significantly reduced at the 24-month evaluation. It must be emphasized that while EDSS evaluation was performed by four blinded neurologists, the assessment of exacerbations was monitored by treating physicians not blinded to study treatment (see Patients and methods). The unblinded assessment of exacerbations suggests a potential systematic bias concerning treatment efficacy (false positive, type I error) [23]. This type of error, however, is a crucial element when the EDSS score must be assigned, while it seems to be less relevant in recording objective neurological findings such as determining that an exacerbation has taken place.

Although a previous open-label trial with MTX in progressive MS indicated that MTX did not suppress clinical and MRI evidence of disease progression [22], the present study, which is the first controlled trial with MTX in RRMS patients, confirms preliminary reports from two uncontrolled pilot studies showing that MTX may reduce clinical activity in MS [8, 13]. It is therefore important to compare our clinical MTX results with other immunosuppressive or immunomodulator trials. Several immunosuppressive agents such as cyclophosphamide, cyclosporine or total lymphoid irradiation have been extensively tested in progressive MS, with controversial results [3, 18, 27, 28, 30, 32, 33], and the few trials that have been performed in RRMS have shown a modest therapeutic benefit at safe doses [14, 27]. Other drugs such as azathioprine, interferon beta (IFN_β) and copolymer 1 (Cop 1) have been tested mainly in RRMS patients [11, 12, 34]. Although these trials involved patients similar to those in the present MTX trial, they differed slightly in design, execution, and primary study outcome measures, which means care should be taken in making direct comparisons. However, data from all these studies address the effect of the drugs on annualized attack rates whose reduction ranges from 25% to 33% in the treated groups compared with the placebo groups. In this regard, MTX seems to have a greater effect on the attack rate. Moreover, though neither azathioprine, IFN_β nor Cop 1 significantly influenced the progression of the disease in terms of disability [11, 12], with MTX there seems to be a significantly smaller number of patients with confirmed progression of the disease as measured by a one point increase on the EDSS scale.

The MRI data of the present study do not completely support the clinical findings. We observed only a trend towards a reduction in the number of new lesions on T2-weighted images. The lack of a total lesion volume evaluation on T2-weighted images, which is the most appropriate measurement for long-term studies, and the use of dif-

ferent MRI imagers, which might be a significant source of variation for lesion measurements, appear to be the major limitations of the present study and might explain the apparent incongruity between clinical and MRI results. A significant effect of MTX on MRI activity has, in fact, been reported in three recent studies. Krapf et al. [15] performed a serial gadolinium (Gd)-enhanced MRI study in ten patients with MS treated with MTX at a dose of 12 mg/m² surface area every 3 months over a 1-year period. They found that the total number of Gd-enhancing lesions diminished from 169 at baseline to 10 after 1 year, and to 5 after 2 years. This reduction and the percentage of follow-up MRI studies showing no Gd enhancement were more pronounced than in other MRI studies on the natural course of MS. Bastianello et al. [1] reported MRI findings from a subgroup of patients in our trial (25 patients: 13 MTX and 12 placebo) based on a serial Gd-enhanced MRI study at 0, 2, 4, 6 and 12 months from the beginning of the study. The MTX group showed a trend towards a 41%, 45% and 60% reduction of new, enlarging and enhancing lesions respectively. Finally, Edan et al. [5] randomized 42 MS patients with a very active disease, as assessed by clinical and MRI criteria, to receive either MTX (20 mg IV monthly) and methylprednisolone (1 g IV monthly) or methylprednisolone alone over 6 months. A highly significant increase was observed in the percentage of patients with no new enhancing lesions in the MTX group compared with the group receiving only steroid.

According to extensive oncological experience, the tolerability and long-term toxicity of MTX seem to compare favourably with that of other cytostatic agents [20]. The major dose-limiting toxicity is a predictable and reversible myelosuppression, manifested as leukopenia and neutropenia, with minimal effects on the platelet and red cell series. Mild to moderate nausea, vomiting, alopecia, amenorrhoea and stomatitis occasionally occur, but most patients experience limited or no non-haematological adverse effects [31]. In our study, no serious side effects were observed among patients treated with MTX. Moreover, five women showed secondary amenorrhoea but all resumed their menstrual cycle spontaneously. As potential cardiotoxicity represents the primary long-term adverse reaction, MTX therapy is contraindicated in patients with cardiovascular risk factors. Our population showed no alteration of the electrocardiographic findings. No significant differences were found in left ventricular function

between the two groups [4]. In particular, patients treated with a cumulative dose of 96 mg/m² MTX showed no reduction in left ventricular ejection fraction or fractional shortening, or modifications in Doppler indices of left ventricular systolic and diastolic function during follow-up. These findings have several possible explanations. Firstly, as suggested from previous reports, we studied only young patients who had not been exposed to previous cardiotoxic therapies or mediastinal radiation, and had no pre-existing cardiac pathology, reducing the risk of cardiotoxic effects of MTX [26, 29]. Secondly, it should be emphasized that we used low doses of MTX with an intermittent infusion schedule. This pharmacological schedule is different from those used in previous reports where the drug was used to obtain an antineoplastic effect [2].

In conclusion, our data suggest that MTX might be effective in reducing disease activity, both by decreasing the number of exacerbations and by slowing the clinical progression sustained for most patients 1 year after the end of treatment. However, further larger studies are needed to demonstrate that MTX may stabilize patients with frequent exacerbations by halting the progression of the disability. At present, even if MTX appears to be fairly well tolerated, repeated courses of MTX cannot be given because of concerns about possible cumulative cardiac toxicity, and it should be restricted to patients with frequent exacerbations and rapid disease progression. A further study specifically designed to compare the effectiveness of alternative therapies for RRMS such as MTX versus Cop1 or IFNB would be welcome.

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