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ORIGINAL COMMUNICATION

Effect of natalizumab on conversion of gadolinium enhancing lesions to T1 hypointense lesions in relapsing multiple sclerosis

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Abstract *Background* Natalizumab, a humanized monoclonal anti-adhesion molecule antibody, reduces the frequency of new gadolinium (Gd) enhancing lesions and relapses in multiple sclerosis (MS). Its effect on evolution of new Gd enhancing lesions to T1 hypointense lesions is unknown. *Methods* 213 patients were randomized to receive 3 mg/kg or 6 mg/kg natalizumab or placebo monthly for 6 months and then followed for a further 6 months. A subset of patients who had one or more new gadolinium enhancing lesions from Month 0 to Month 6 and available electronic data were analysed. Each new Gd enhancing lesion that developed during treatment (months 1–6) was investigated for conversion to a new T1 hypointense lesion at month 12. Lesions were classified as large or small if their cross-sectional area was greater or less than 20 mm^2. Because of the similarity of both doses of natalizumab on the frequency of new Gd enhancing lesions, the two natalizumab arms were combined in all analyses. *Results* Compared with the placebo group, the natalizumab group exhibited significant decreases in: (i) the proportion of patients with new Gd enhancing lesions that evolved to T1-hypointense lesions (10/38 [26 %] versus 27/40 [68 %]; $p < 0.01$); (ii) the proportion of patients who developed large T1 hypointense lesions (2/38 [5 %] versus 16/40 [40 %]; $p < 0.01$); (iii) the proportion of new Gd enhancing lesions that became T1 hypointense (11/75 [15 %] versus 118/466 [25 %]; $p = 0.045$); (iv) the mean proportion per patient of new Gd enhancing lesions that converted to T1-hypointense lesions (0.15 versus 0.28; $p = 0.005$), and (v) the odds ratio (OR) of converting from Gd enhancing to T1-hypointense lesions (OR = 0.48; 95 % CI = 0.24, 0.94, $p = 0.031$)). *Conclusion* Natalizumab significantly suppresses the evolution of new Gd enhancing to T1-hypointense lesions. This may reflect several mechanisms including reduced cell migration across the blood brain barrier, reduced T cell activation within lesions, an inhibitory effect on subsequent axonal damage within the new central nervous system lesion, and a reduced likelihood of recurrent lesion inflammation.

Key words Natalizumab · relapsing · multiple sclerosis

Introduction

Serial MRI in patients with relapse onset forms of multiple sclerosis (MS) has shown that asymptomatic new lesions develop about 5 to 10 times more frequently than do clinical relapses [17, 21, 25]. The new lesions usually display gadolinium (Gd) enhancement, indicating that there is breakdown of the blood-brain barrier (BBB) [12]. The phase of Gd enhancement lasts about 2–6 weeks [11] and several studies using post mortem or biopsy material have demonstrated a correlation of enhancement with histological signs of activity including macrophage infiltrates, perivascular lymphocytes and myelin breakdown [6, 14]. Follow up MRI shows that most enhancing lesions result in an area of permanent high signal on T2-weighted scans indicating that chronic structural changes have occurred [4]. Whereas most lesions display hypointensity on T1-weighted images during the acute enhancing phase, a minority (about 14–41 %) become T1 hypointense with follow up [3, 4, 7, 23]. Such chronically hypointense lesions are associated with more extensive axonal loss than the larger proportion of T2 lesions which become T1-isointense [1, 22, 24]. Axonal loss is likely to be an important substrate of irreversible disability in MS, and there has been interest in following the accumulation of T1 hypointense lesions in studies of natural history and therapeutic intervention [2, 3, 7, 20]

The evidence that BBB breakdown is a consistent feature of new lesions suggests that migration of immunologically active white cells from the blood into the brain might be an important event in leading to foci of demyelination and ultimately axonal damage or loss [15]. A question arising is whether therapy that specifically interrupts such cell migration might prevent the formation of new lesions [26]. In a recent 6 month, placebo-controlled trial of natalizumab, a humanized monoclonal antibody directed against the $\alpha 4$ integrin adhesion molecule, natalizumab reduced the mean frequency of new enhancing lesions per patient by about 90 % and the number of relapses by about 50 % [16].

Because of inhibition of cell migration across the BBB, it is theoretically possible that when new lesions form in patients receiving natalizumab, there is a quantitative reduction in the severity of inflammation which in turns leads to less permanent axonal damage and loss. We now report our effort to investigate this possibility by determining the frequency with which new enhancing lesions became T1 hypointense lesions after at least 6 months of follow up in patients who had previously received up to 6 monthly infusions of natalizumab or placebo.

Patients and methods

Two hundred and thirteen patients with relapsing MS from 26 clinical centers in the United States, Canada and the United Kingdom were recruited and randomized into a double-blind placebo-controlled trial of natalizumab [16].

The institutional review board or central and local ethics committee approved the protocol for each participating site. Patients gave written informed consent. Inclusion criteria were: an Expanded Disability Status Scale (EDSS) [13] of between 2 and 6.5; Poser [19] criteria clinically definite or laboratory supported definite MS, either relapsing remitting or secondary progressive; a history of at least two relapses within the previous two years; a minimum of three lesions on T2-weighted brain MRI; and aged between 18 and 65 inclusive. Patients were excluded if they received immunosuppressive or immunomodulatory treatments within the preceding three months or experienced a relapse or received systemic corticosteroids within the 30 days prior to enrolment.

Patients were randomized equally into one of three treatment groups: 3 mg/kg natalizumab, 6 mg/kg natalizumab, or placebo. Patients received 6 intravenous infusions at 28 day intervals and then had 6 months of safety follow-up. The investigator, all other study personnel, and patients were blinded to treatment assignment.

MRI acquisition protocol

Pre-contrast T1-weighted spin echo (SE) images were acquired during the screening phase at month minus one, immediately prior to treatment at month 0, one month following the last treatment at month 6 and during the follow up phase at month 12 using the following parameters: repetition time (TR) = 500–700 ms; echo time (TE) = 5–25 ms.

Pre-contrast Proton Density (PD) and T2-weighted SE scans were acquired during the screening phase (month –1), immediately before each treatment (month 0–5), one month following the last treatment (month 6) and during a safety follow-up at months 9 and 12, using the following parameters: TR = 2–2.5 s; short echo time TE = 30–40 ms; long echo TE = 80–100 ms. Also at each of these visits, a T1-weighted SE was acquired 5 to 7 minutes after the administration of 0.1 mmol/kg gadolinium diethylenetriamine pentaacetate [Gd-DTPA] using the same parameters as the corresponding pre-contrast scan. For all of the MRI sequences, 46 axial oblique, contiguous, interleaved slices were acquired with a matrix of 256 × 256 and a slice thickness of 3 mm. Repositioning for all follow up MRI scans was achieved by using a protocol based on identification of standardized anatomical landmarks [10].

MRI analysis

All MRI studies performed at the individual centers were archived onto hard copy film and electronic media and transported to the MRI Analysis Center (Institute of Neurology, University College London, Queen Square, London, UK), where analyses were performed by staff blinded to the clinical details.

On hard copy scans, two experienced observers identified and counted the number of new and persisting T1 gadolinium enhancing lesions at months 0, 1, 2, 3, 4, 5, 6, 9 and 12 (results previously published) [16], as well as new and enlarging T2 hyperintense lesions and new T1 hypointense at months 6 and 12. The electronic form of the data was used to quantify T2 lesion volume and T1 hypointense lesion volume at months 0, 6 and 12 [5].

Following completion of the clinical study, an independent observer blinded to the study treatment retrospectively re-examined monthly MRI scans using electronic and hard copy data and located each new gadolinium enhancing lesion at months 0, 1, 2, 3, 4, 5, 6. Because a previous study showed that large Gd enhancing lesions were

more likely than smaller ones to become T1 hypointense [3], we divided lesions as large or small for additional analysis. This was done on the electronic images according to their largest area on a single slice. Large lesions were > 20 mm^2 and small ≤ 20 mm^2 [18]. This area approximates to a lesion diameter of 5 mm, a criterion that was used to separate large and small lesions in the previous study [3]. The site of each new Gd enhancing lesion seen at months 0–6 was then examined on the month 12 pre-contrast T1-weighted SE image to its determine whether it appeared as either T1 hypointense or isointense. It was defined as T1 hypointense lesion if it was an area of low signal intensity compared with the surrounding white matter in a region in which there was a high signal lesion on the corresponding month 12 T2-weighted image. Otherwise it was classified as T1 isointense. The T1 hypointense lesion at month 12 was confirmed as being a consequence of the previous co-located new gadolinium enhancing lesion by establishing that it was not present on the scan obtained one month before the initial enhancing lesion had appeared. The lesion at month 12 was also verified as not enhancing by reference to the post-contrast T1-weighted scan obtained at the same visit. T1 hypointense lesions were classified as small or large lesions using the same cut off of 20 mm^2. The numbers of small and large new Gd enhancing lesions during month 1 to 6 together with the corresponding number of new small and large T1 hypointense lesions at month 12 scan were recorded for each patient with available electronic data.

Statistical Analysis

For comparisons with placebo, we decided to combine both natalizumab arms in to a single group. This improved the power of the statistical analyses as a larger number of Gd enhancing lesions were available for analysis in natalizumab treated patients (bearing in mind that the overall frequency [per patient] of such enhancing lesions was about 90 % less in natalizumab treated patients than in the placebo patients). We felt that this approach was biologically sound because: (i) similar efficacy had already been shown for each natalizumab arm with respect to the frequency of enhancing lesions and relapses [16]; (ii) pharmacokinetic studies showed near full saturation of the α4 receptor with both doses of natalizumab [16], and (iii) inspection of the individual treatment group data suggested a trend to decreased evolution from Gd enhancing to T1 hypointense lesions in both groups.

Patients with one or more new Gd enhancing lesions from Month 0 to Month 6 and available electronic data were analysed. The number and percentage of patients with new T1 hypointense lesions formed from new Gd enhancing lesions at baseline (month 0) and during the treatment period from months 1 to 6 were calculated and compared between placebo and treated groups using the Fisher's Exact Test. The proportions of new Gd enhancing lesions converted to T1 hypointense lesions were calculated at baseline (month 0) and during the treatment period from Month 1 to Month 6 for each patient. Comparisons of the distribution of these proportions between the placebo and treated groups were made using the Wilcoxon rank sum test.

A logistic regression model with generalized estimating equations (GEE), which adjusts for correlations of lesions within the same patient, was used to compare the probability of new Gd enhancing lesions converting to T1 hypointense lesions in the combined natalizumab treated group compared with the placebo group during the treatment period from months 1 to 6. Odds ratio (OR) of conversion to T1 hypointense lesions was computed using the placebo group as the reference group.

All statistical tests were two-sided and carried out at the 5 % level of significance.

Results

Of the whole cohort of 213 patients, 71 were randomized to receive placebo and 142 natalalizumab. Of this total, 86 patients had one or more new Gd enhancing lesions from months 1–6. Seventy eight/86 (40 placebo and 38 Natalizumab) were included in the final analysis because they had available all electronic data and hard copy data from all scans obtained from months 0–6 and 12. Eight/86 patients (6 placebo, 2 natalizumab treated) with new Gd enhancing lesions from months 1–6 had missing data such that they had to be excluded from the analyses, except in the logistic regression/GEE analysis, where data for three more patients (one placebo, 2 natalizumab treated) who had only one missing scan were also included, as GEE can accommodate data with some missing values.

The clinical demographic features of the subgroup of 78 were similar to those of the whole study cohort (Table 1; [16]). Compared with the whole cohort, patients in the study subgroup were more likely to display Gd enhancing lesions on the two baseline scans, especially those who went on to receive natalizumab. This finding is expected since subjects with enhancing lesions on single or baseline scans are known to be more likely to exhibit enhancing lesions during the next few months.

The baseline (month 0) number of new Gd enhancing lesions, and the proportions of enhancing lesions at month 0 which converted to T1 hypointense lesions, were similar in both placebo and natalizumab groups (Table 2).

The total number of new Gd enhancing lesions during the treatment period (months 1–6 scans) was 466 in the placebo group, and 75 in the combined natalizumab group (Table 3). The corresponding total number of new T1 hypointense lesions at month 12 (that evolved from new Gd enhancing lesions between months 1 and 6) was 118 in the placebo group and 11 in the combined natalizumab group; the occurrence of 3 or more new T1 hypointense lesions was only observed in the group of patients treated with placebo (Table 3).

At month 12 the number (%) of patients with new T1 hypointense lesions formed from new Gd enhancing lesions during the treatment period from months 1 to 6 was 27/40 (68 %) in the placebo and 10/38 (26 %) in the combined natalizumab group ([Table 3]; Figure). The mean proportion per patient of new Gd enhancing lesions during the treatment period from months 1–6 which converted to T1 hypointense lesions at month 12 was 0.28 in the placebo group, and 0.15 in the combined natalizumab group ($p = 0.005$; Table 3).

The Odds Ratio (OR) of conversion to T1 hypointense lesions, comparing the natalizumab group combined (40 patients) with the placebo group (41 patients) showed a reduced probability of new Gd enhancing lesion during the treatment period from months 1 to 6 converting to T1 hypointense lesions at month 12

Table 1 Demographic and baseline MRI characteristics of Patients Evaluated (n = 78)

Characteristic	Natalizumab	
	Placebo (N = 40)	3.0 mg/kg + 6.0 mg/kg combined (N = 38)
Age, years		
Mean	39.8 (8.27)	42.7 (8.03)
Range	22–54	30–59
Gender N (%)		
Male	13 (32.5)	12 (31.6)
Female	27 (67.5)	26 (68.4)
MS category N (%)		
Relapsing remitting	28 (70.0)	27 (71.1)
Secondary progressive	12 (30.0)	11 (29.0)
EDSS		
Mean	4.3	4.2
Range	2.0–6.5	1.5–6.5
Disease duration (Years)		
Mean	9.4	11.6
Range	1–32	1–33
Number of relapses (in past 2 years)		
Mean	3.1	2.9
Range	2–12	2–6
Time since last relapse (Months)		
Mean	6.2	6.6
Range	2–14	2–13
Screening T1-weighted MRI (Month –1)		
Number (%) of scans with one or more Gd-enhancing lesion (s)	21 (53)	23 (61)
Number of Gd-enhancing brain lesions		
Mean	1.3	1.9
Range	0–11	0–14
Baseline T1-weighted MRI (Month 0)		
Number (%) of scans with one or more new Gd-enhancing lesion (s)	14 (35)	25 (66)
Number of new Gd-enhancing brain lesions:		
Mean	1.1	1.8
Range	0–10	0–10

EDSS expanded disability status scale

(OR = 0.48; 95 % Confidence Intervals = 0.24, 0.94, p = 0.031). The difference remained significant when the analysis was adjusted to include disease subgroups (relapsing remitting and secondary progressive; p = 0.035)

The proportions of patients with large new Gd enhancing and T1 hypointense lesions were lower in the natalizumab than placebo group, but the reduction appeared greater for T1 hypointense lesions (Table 4).

Discussion

In the placebo group of this study, 25 % of new Gd enhancing lesions had evolved in to areas of T1 hypointensity at month 12 follow up. This proportion is well within the range (14–41 %) of such an evolution reported in other studies [3, 4, 7, 23]. This relatively wide range is likely to reflect differences in the length of follow up, the appearance (and limited tissue contrast) of T1-weighted spin echo images, and the visual threshold used for determining an area of T1 hypointensity.

Two previous studies have investigated the evolution of new Gd enhancing to T1 hypointense lesions in the context of a placebo-controlled clinical trial [3, 7]. In one study, of patients with relapsing remitting MS, treatment with glatiramer acetate was associated with a decreased likelihood of Gd enhancing lesions evolving to T1 hypointensity (15 % versus 31 %; [7]). In another study of beta interferon-1b in secondary progressive MS, a similar frequency of such a lesion evolution was observed in both the beta interferon and placebo groups (13.4 % versus 14 %; [3]).

Table 2 Baseline (month 0) number of new Gd lesions, and number and proportion converted to T1 hypointense lesions at month 12

	Placebo (n = 40)	Natalizumab Combined (n = 38)
Number of new Gd lesions per patient		
N	40	38
Mean (sd)	1.1 (2.28)	1.8 (2.22)
Median,	0	1
Min. max.	0, 10	0, 10
Number of new Gd converted to T1 hypointense lesions per patient		
N	40	38
Mean (sd)	0.3 (0.60)	0.4 (0.68)
Median,	0	0
Min. max.	0, 2	0, 3
Proportion of Gd lesions converted to T1 hypointense lesions per patient		
N	14	25
Mean (sd)	0.31 (0.376)	0.27 (0.348)
Median,	0.16	0.13
Min. max.	0, 1	0, 1

Note: N are smaller for proportion converted to T1 hypointense lesions, because for those patients with no Gd lesions, the proportion is set to be missing

Although the total number of new Gd enhancing lesions available for analysis was much less in the natalizumab groups than the placebo group, there were still sufficient numbers of lesions when the two natalizumab arms were combined to detect significant differences in outcomes between the treated and placebo groups. The analyses collectively provide evidence that treatment with natalizumab is associated with a significant reduction not only in the frequency with which new Gd enhancing lesions form but also in the frequency with which such lesions become areas of T1 hypointensity. The data suggest that the odds of Gd enhancing lesions becoming T1 hypointense are reduced by about 50 % in patients treated with natalizumab, although the confidence intervals around this estimation are wide.

Several potential mechanisms may have modified the evolution from enhancing to T1 hypointense lesion. The first (and most likely) mechanism is an effect of natalizumab to inhibit adhesion between circulating white cells and cerebral endothelial cells (by blocking the interaction of α4 integrins on lymphocytes and monocytes cells and vascular cell adhesion molecule 1 [VCAM-1] on cerebral endothelium) [8, 9], with a consequential decrease in the cell migration into new lesions that does occur. Such lesions may thereby contain quantitatively less severe inflammatory mediators of tissue damage and a reduction in the extent of acute axonal damage will reduce the likelihood of lesions subsequently becoming T1 hypointense.

Secondly, there could be a therapy-induced reduction T cell activation within the lesion or an inhibitory effect on subsequent axonal damage within the lesion. Such effects occurring within the brain might be expected to result in a greater decrease in T1 hypointense than Gd enhancing lesions. Although the overall marked decrease in frequency of both types of lesions is consistent with a predominant therapeutic action at the BBB, the suggestion that large T1 hypointense lesions are reduced more markedly than large Gd enhancing lesions (Table 4) could indicate an additional effect within the brain. Thirdly, there may have been a greater tendency for lesions in the placebo group to undergo recurrent episodes of Gd enhancement (and by implication recur-

Table 3 Number and percentage of patients with new T1 hypointense lesions at month 12 in patients with at least one new Gd enhancing lesion from months 1 to 6

	Placebo	Natalizumab Combined	p-value
Total number of patients with new Gd lesions months 1–6	40	38	
Number (%) patients with T1 hypointense lesions	27 (68 %)	10 (26 %)	$p < 0.01$[a]
Number of patients with T1 hypointense lesions			
0	13	28	
1	9	9	
2	4	1	
> = 3	14	0	
Total number of T1 hypointense lesions/ Gadolinium enhancing lesions (%)	118/466 (25 %)	11/75 (15 %)	$p = 0.0445$[b]
Mean proportion of new Gd-enhancing lesions converted to T1 hypointense lesions (patient level)			
mean median	0.28, 0.25	0.15, 0.0	$p = 0.0051$[c]

[a] Fisher's Exact Test, comparing treated combined versus placebo
[b] Chi-square test, comparing treated combined versus placebo
[c] Wilcoxon Rank Sum Test, comparing treated combined versus placebo

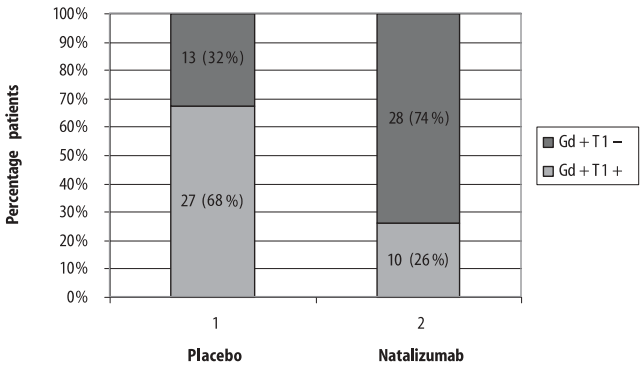

Figure Classification of patients with new Gd enhancing lesions from months 1–6 and the presence or absence of new T1 hypointense lesions at month 12

Gd + T1– Number of patients with new Gd enhancing lesions from months 1–6 but no new T1 hypointense lesions at month 12

Gd + T1 + Number of patients with new Gd enhancing lesions from months 1–6 and new T1 hypointense lesions at month 12

rent inflammation) that in turn could increase the likelihood of producing a T1 hypointensity [23]. In general, however, areas of re-enhancement are seen less often than areas of de novo enhancement [17, 23].

The present study was not powered to definitively address the effect of treatment on relapse related outcomes and over the 6 months of the study there was no significant change in disability progression, measured using the Kurtzke expanded disability status scale [13], in either the treated or placebo group. However, the decrease in evolution from enhancing to T1 hypointense lesions might be expected to have, as its clinical corollary, a reduction in persistent deficits that result from relapses. The larger and longer duration phase III trials of natalizumab that are in progress will address clinical outcomes in a more definitive manner.

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Table 4 Frequency of large new lesions (> 20 mm^2) in natalizumab and placebo groups

	Placebo	Natalizumab Combined	p-value
Total number of patients with new Gd lesions month 1–6	40	38	
Number (%) patients with large T1 hypointense lesions month 12	16 (40 %)	2 (5 %)	$p < 0.01$[a]
Number of large T1 hypointense lesions	46	2	
Number (%) patients with large new Gd enhancing lesions months 1–6	25 (63 %)	19 (50 %)	$p = 0.361$[a]
Number of large new Gd enhancing lesions month 1–6	112	26	

[a] Fisher's Exact Test, comparing treated combined versus placebo

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