Effects of Interferon Beta-1b on Black Holes in Multiple Sclerosis Over a 6-Year Period With Monthly Evaluations

Francesca Bagnato, MD; Shiva Gupta, BA; Nancy D. Richert, MD, PhD; Roger D. Stone, BS; Joan M. Ohayon, CRNP; Joseph A. Frank, MD; Henry F. McFarland, MD

Background: Chronic, hypointense black holes (BHs) are recognized as a sign of permanent damage in patients with multiple sclerosis. Although the effects of interferon beta-1b in reducing the formation of new BHs are established, it is not clear whether the drug may reduce BH duration after these lesions are formed.

Objective: To analyze the effects of interferon beta-1b in reducing the duration of T1 BHs in patients with multiple sclerosis.

Design: Patients were clinically assessed and imaged monthly over a 36-month natural history phase and 36-month therapy phase. Numbers of contrast-enhanced lesions and newly formed BHs were counted on each scan. Each BH was counted until it was no longer seen.

Setting: Outpatient service of the Neuroimmunology Branch at the National Institutes of Health, Bethesda, Md.

Patients: Six patients with relapsing-remitting multiple sclerosis were included. One patient did not form any BHs during the therapy phase. Analyses were performed on the remaining 5 individuals.

Interventions: Interferon beta-1b at the dosage of 8 million international units every other day.

Main Outcome Measures: Number and duration (in months) of newly formed BHs.

Results: Rate of BH accumulation decreased with treatment ($P = .01$), but Kaplan-Meier models revealed that the duration of BHs did not shorten ($\chi^2 = 2.47$, $P = .12$).

Conclusions: Interferon beta-1b reduces the frequency of new BH formation but does not appear to decrease their duration in time. Analyses with larger patient cohorts are needed to confirm these preliminary findings.

Arch Neurol. 2005;62:1684-1688

INTERFERON BETA (IFNβ) reduces formation of contrast-enhanced lesions (CELs)\(^2\)\(^3\) and T1 hypointense black holes (BHs) on magnetic resonance imaging (MRI)\(^4\) in patients with multiple sclerosis (MS).

Questions remain regarding the ability of the drug to shorten the duration of BHs, the formation of permanent detrimental lesions may be prevented, ultimately exerting a neuroprotective effect.

For editorial comment see page 1666

In a previous study, we observed that the life span of BHs did not change in a cohort of 12 patients imaged monthly for 18 months before therapy and 18 months during IFNβ-1b treatment.\(^4\) Because (1) the findings were not significant (ie, $P = .26$), although a trend toward a positive effect in shortening BH duration was seen to be operating by IFNβ-1b; (2) a small number of lesions was detected during the therapy phase; and (3) a relatively large proportion of censored observer.

CME course available at www.archneurol.com

BHs after they have formed. This is clinically relevant because BHs of shorter duration are believed to be associated with the presence of transient edema, and chronic, persisting BHs reflect areas of irreversible axonal loss and permanent damage.\(^5\) Hence, by shortening the duration of

©2005 American Medical Association. All rights reserved.

Downloaded From: by a Non-Human Traffic (NHT) User on 11/06/2018
vations was present during the short-term follow-up, further investigations were warranted.8

This study is an attempt to extend these preliminary findings in a cohort of patients with MS followed for a longer period. For 6 patients with relapsing-remitting (RR) MS, we analyzed MRIs obtained during the 36-month periods immediately before and after the start of IFNβ-1b therapy. We describe the accumulation of newly formed BHs in individual patients during a longer natural history phase (NHP) and therapy phase (TP). We also compare the duration of new BHs arising during the TP with those originating during the NHP.

METHODS

PATIENTS AND STUDY DESIGN

This retrospective study was approved by the intramural research board of the National Institute of Neurological Disorders and Stroke (Bethesda, Md) and performed at the National Institutes of Health (Bethesda). Each patient signed an informed consent. Six female patients with RRMS7 were sequentially enrolled. We excluded patients who had previously received immunomodulatory or immunosuppressive drugs, except intravenous methylprednisolone at 1 g per day for 3 to 5 days or oral prednisone taper for a clinical relapse. Patients were clinically examined by rating disability using the Expanded Disability Status Scale (EDSS)9 and imaged monthly for 71 months (ie, 72 evaluations) without missing any clinical examination or MRI. Sustained change in the EDSS score was defined as any change of 1 or more for an EDSS score of 5.0 or lower or a change of 0.5 or more for an EDSS score of 5.5 or more confirmed on at least 2 examinations 3 months apart.9,10

MRI ACQUISITION AND EVALUATION

A total of 432 MRI scans were obtained. Standard precontrast and postcontrast MRIs (gadopentate dimeglumine, 0.1 mmol per kilogram of body weight) were performed at 1.5 T (General Electric Medical Systems, Milwaukee, Wis).11 One of us (F.B.) recorded the numbers of CELs and BHs. This procedure was repeated 3 times for each patient until a coefficient of variation of less than 2% was reached. Black holes were defined as hypointense regions visible on nonenhanced T1-weighted images with corresponding hyperintensities on T2-weighted images, but without enhancements on postcontrast MRIs. Black holes already present at the first scan gave the number of preexisting BHs. These procedures were used for the subsequent statistical analyses performed in this article. Each new BH was tracked until it was no longer visible. No BHs occurred without any evidence of visible CELs or separated by a gap after the cessation of the enhancement during the 36 months of observation.

New BH counts during the NHP and TP were performed separately. That is, lesions visible at the end of the NHP were no longer followed and therefore not tracked into the TP.

NEUTRALIZING ANTIBODIES

Neutralizing antibodies (NABs) were measured every 2 months for the first 24 months of therapy and yearly afterwards by the means of the Mixovirus-A protein inhibition assay from Berkeley Laboratories (Montville, NJ).12 Patients were considered to be NAB+ when NAB titer of 1:20 or more were detected in at least 2 consecutive samples.

STATISTICAL ANALYSIS

For the first 2 types of statistical analyses described here, each patient’s data were considered separately because of heterogeneity among patients. Accumulation of new BHs and comparisons in the accumulations of new BHs over the NHP and TP were performed by a linear regression analysis. The corresponding slopes of these linear relations were evaluated to determine whether the monthly numbers of new BHs increased over time within the NHP and TP. Further analysis of these slopes described whether the rate of accumulation of new BHs during the NHP was significantly higher than during the TP. For these 2 analyses, natural logarithmic transformation of the month of the NHP or TP was applied to linearize the nonlinear regression relation between the month of the NHP or TP and the accumulation of BHs. The third analysis consisted of a Kaplan-Meier survival model and log rank test to detect a potential difference between the duration (measured in months) of new BHs during the NHP vs the TP. For this analysis, data from all individuals were combined. Reported P values were based on 2-tailed statistical tests, with a significance level of .05. The statistical analyses were performed using DataDesk (Data Description Inc, Ithaca, NY) version 6.0 (for Windows) for the slope analyses and StatView (SAS, Cary, NC) version 5.0 for survival analyses and log rank tests.

RESULTS

CLINICAL OUTCOME

At baseline, the age range was 32 to 42 years; the EDSS score range, 1.5 to 3.5; and the disease duration range, 1 to 9 years. The number of CELs ranged from 1 to 5, and the number of preexisting BHs ranged from 3 to 18.

Over the study course, 1 patient (patient 3) had a sustained change in EDSS score during both the NHP (ie, from 3.0 to 6.0) and the TP (from 6.0 to 6.5). Two patients showed increased EDSS scores exclusively over the TP. The EDSS score of patient 2 increased from 1.5 to 3.0, and patient 4 showed an increased EDSS score from 2.0 to 4.5. The EDSS scores of the remaining 3 patients (patients 1, 5, and 6) did not change. The number of clinical exacerbations and MRI outcomes of individual patients during the NHP and TP of the study are reported in Table 1. One patient (patient 6) did not have any CELs or new BHs over the TP and was excluded from the statistical analyses reported here.

IFNβ-1b EFFECT IN FORMATION AND PERSISTENCE OF NEW BHs

The number of accumulating new BHs and natural logarithm of the month were linearly related (P = .001) for each individual within the NHP (R2 range, 58.4%-85.3%) and TP (R2 range, 35.7%-86.1%). Specifically, the number of new BHs increased (P = .001) for each individual within the NHP and TP. However, for each patient, the rate of accumulation of new BHs during the TP was significantly lower than during the NHP (Table 2, Figure 1).

A total of 156 new BHs were identified during the NHP and 31 during the TP. 49.7% of the NHP observations and 38.9% of the TP observations were censored. That
is, a large percentage of the BHs during the NHP and TP persisted to the end of the observation periods, and thus, these BHs were assumed to last to the end of the observation phases. Kaplan-Meier analysis revealed that the duration of new BHs arising during the TP was not shorter than the duration of BHs arising during the NHP ($\chi^2 = 2.47$, $P = .12$; Figure 2).

**Table 1. Clinical Exacerbations and MRI Outcomes of Individual Patients During the NHP and TP**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Exacerbations (Absolute No.)</th>
<th>CELs, Mean ± SD, No.</th>
<th>New BHs, Mean ± SD, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHP</td>
<td>TP</td>
<td>NHP</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3.5 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2.2 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5.7 ± 4.9</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
<td>3.1 ± 2.3</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0</td>
<td>3.4 ± 2.8</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

**Table 2. Accumulation of New BHs During the NHP and TP: Individual Comparisons Between Linearized Slopes of the NHP and TP**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>NHP Slope, 1/ln (mo)</th>
<th>TP Slope, 1/ln (mo)</th>
<th>$F_{1,6}$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.12</td>
<td>0.39</td>
<td>63.9</td>
<td>$&lt;.001$</td>
</tr>
<tr>
<td>2</td>
<td>5.42</td>
<td>0.33</td>
<td>171.3</td>
<td>$&lt;.001$</td>
</tr>
<tr>
<td>3</td>
<td>11.61</td>
<td>0.90</td>
<td>10.4</td>
<td>$&lt;.005$</td>
</tr>
<tr>
<td>4</td>
<td>2.36</td>
<td>0.82</td>
<td>7.4</td>
<td>$&lt;.01$</td>
</tr>
<tr>
<td>5</td>
<td>7.71</td>
<td>0.92</td>
<td>107.1</td>
<td>$&lt;.001$</td>
</tr>
</tbody>
</table>

Abbreviations: BHs, black holes; CELs, contrast-enhanced lesions; MRI, magnetic resonance imaging; NHP, natural history phase; TP, therapy phase.

**NAB OCCURRENCE**

Two patients developed NABs during therapy. Patient 3 had transient low titers (eg, <1:100) at month 20 of therapy. Patient 4 had a sustained NAB seroconversion, beginning during the third month of therapy. Specifically, titers increased up to 1:200 at month 9 of therapy,
The results of the present article are based on the evaluation of serial MRIs of 6 patients. The sample size is small, but the length of the longitudinal follow-up (ie, 72 months) represents a robust and valid data set for describing the course of MS during both NHPs and TPs. We demonstrate that new BHs may significantly accumulate over time even when IFNβ-1b is administered. However, repeated administration of the drug did significantly decrease the rate of BH formation, thus protecting the brain tissue from accumulating degenerative lesions. Except for patient 4, a dramatic decrease in the number of CELs was observed in each individual patient, and this might have accounted for the corresponding decrease in the formation of new BHs. In this regard, our findings are similar to those reported in previous studies, which were based on analyses of larger cohorts of patients but with shorter monthly time windows.3,4,13

Given the exceptional behavior seen here in patient 4, additional analyses were performed. The NABs occurred from the early months of therapy in this patient and persisted throughout the study period. Neutralizing antibody formation could account for the lack of decrease in CEL occurrence. However, of the total new CELs, a significant decrease in the proportion of CELs forming BHs was found when comparing NHP vs TP lesions (data not shown). One may hypothesize that NABs did not block the whole amount of IFNβ-1b administered to this patient. Residual drug, if any is present, although unable to reduce the amount of inflammatory activity, might have promoted the formation of less severe inflammatory lesions (as given by the ability of CELs to evolve into BHs) during the TP. The effect of IFNβ in promoting the formation of less aggressive CELs has been previously reported. Repeated administration of IFNβ reduced the proportion of CELs converting into BHs in patients with RRMS14 (as for patient 4) but not in those with secondary progressive MS or mixed populations of patients with RRMS and secondary progressive MS.13,15 A possible explanation for these observations is that the immune factors that allow CELs to arise during the RR phase of MS are less aggressive and allow more prompt repair during the administration of IFNβ than the CELs of patients with progressive disease. However, care needs to be taken in the interpretation of our results (reported on an individual case) with particular respect to the NAB findings. Additional appropriate investigations are warranted.

Amelioration of the disease course was further studied to see if IFNβ could shorten the life span of new BHs. This constituted the primary aim and novel aspect of our study. For this analysis, lesions (ie, BHs) were pooled across patients. Kaplan-Meier analysis showed that the duration of new BHs that arose during the NHP was not shorter than those that arose during the TP. One can postulate that although IFNβ may reduce the frequency of BHs, after the lesion occurs, the drug is not changing the pathological process. However, the significance levels at which differences were detected are not straightforward. Heterogeneity in the number of new BHs for each patient as well as large proportions of censored observations during the study phases may potentially bias these results. Consequently, we cannot distinguish between the possible ability of IFNβ-1b to promote either the formation of less aggressive new BHs or faster recovery from them. Knowing this would be of great importance and would suggest the utility of introducing Kaplan-Meier approaches for lesion survival as additional tools for monitoring drug efficacy. This would allow one to properly establish the role of IFNβ-1b (or any other neuroprotective drug of the central nervous system) for patients with MS. Larger cohorts of patients over longer periods are required to minimize potential bias because of the high heterogeneity of disease expression in individual cases.

Accepted for Publication: March 15, 2005.

Correspondence: Francesca Bagnato, MD, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bldg 10, Room 5B16, 10 Center Dr, Bethesda, MD 20892-1400 (bagnato@ninds.nih.gov).

Author Contributions: Study concept and design: Bagnato, Gupta, Richert, Frank, and McFarland. Acquisition of data: Richert, Stone, Ohayon, and Frank. Analysis and interpretation of data: Bagnato, Gupta, and Richert. Drafting of the manuscript: Bagnato, Gupta, and Frank. Critical revision of the manuscript for important intellectual content: Bagnato, Gupta, Richert, Stone, Ohayon, Frank, and McFarland. Statistical analysis: Bagnato and Gupta. Obtained funding: McFarland. Administrative, technical, and material support: Stone and McFarland. Study supervision: Frank and McFarland.

Acknowledgment: It is a pleasure to record our indebtedness to all the patients.
REFERENCES


