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Disease-modifying drugs for multiple sclerosis and JC virus expression

Craig S. Miller · Sidney A. Houff · Jason Hopper · Robert J. Danaher · Julie A. Gurwell · Yushun Lin · Nubia Vega · Joseph R. Berger

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Abstract Natalizumab-associated progressive multifocal leukoencephalopathy in multiple sclerosis (MS) occurred in two individuals also treated with interferon β1a, raising concerns about the interaction of these disease-modifying agents and leading to the recommendation to avoid their concomitant administration. However, type I interferons are antiviral. Using a real-time quantitative polymerase chain reaction for the detection and quantification of the John Cunningham virus (JCV), DNA in peripheral blood mononuclear cells (PBMCs), and urine in MS patients, we tested the hypothesis that MS disease-modifying drugs (DMD) qualitatively and quantitatively alter JCV prevalence and viral copy numbers. Two hundred thirty-nine patients were enrolled in a cross-sectional study in which blood and urine specimens were collected at a single time and 37 newly diagnosed, treatment-naïve MS patients were enrolled in a longitudinal study in which specimens were obtained at diagnosis and 6 months after treatment initiation. JCV DNA was detected in PBMCs of only two patients (0.07 %), but was commonly detected in the urine (46.8 %) in this population. There was no effect of DMDs on blood or urinary JCV prevalence or viral copy numbers with either glatiramer acetate (Copaxone®) or interferon-β therapy (Avonex®, Betaseron®, or Rebif®). The small number of patients on other therapies precluded meaningful comment about their effects. No obvious effect of the platform DMDs on JCV prevalence was observed even for the interferon-βs.

Keywords JC virus · Multiple sclerosis · Interferon-β · Glatiramir acetate · Progressive multifocal leukoencephalopathy

S. A. Houff · J. A. Gurwell · N. Vega · J. R. Berger
Department of Neurology, University of Kentucky,
Lexington, KY, USA

J. Hopper
University of Kentucky,
Lexington, KY, USA

Y. Lin
Department of Statistics, University of Kentucky,
Lexington, KY, USA

C. S. Miller · R. J. Danaher
College of Dentistry,
University of Kentucky,
Lexington, KY, USA

J. R. Berger (✉)
Department of Neurology, College of Medicine,
University of Kentucky,
Kentucky Clinic L-445, 740 S. Limestone St,
Lexington, KY 40536-0284, USA
e-mail: jrbneuro@uky.edu

Introduction

Progressive multifocal leukoencephalopathy (PML) occurring in patients with multiple sclerosis (MS) was unheard of until the recognition of natalizumab-associated PML in 2005 (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005). Both seminal cases of natalizumab-associated PML also received interferon (IFN)-β1a. The potential risk of PML with the co-administration of IFN-β with natalizumab remains uncertain. As of November 1, 2011, 181 natalizumab-associated PML have occurred in the absence of concomitant IFN-β therapy (BiogenIdec 2011). Current risk estimates for PML with natalizumab are the same or higher than that based on the pivotal trials (Yousry et al. 2006; BiogenIdec 2011).

The type 1 interferons, the multiple IFN-αs and both IFN-βs, are known to have an antiviral effect (Biron 1999; Kawai and Akira 2006). Indeed, they were first recognized because of their antiviral properties (Biron 1999) and a sustained elevation in their systemic levels occurs during

viral infection (Biron 1999). Among their antiviral effects are induction of maturation of dendritic cells, facilitation of viral antigen presentation, induction of CD8+ T cell responses, stimulation and recruitment of lymphocytes and monocytes to inflamed sites, upregulation of hundreds of effector molecules (Kawai and Akira 2006), and rendering cells refractory to viral-induced protein synthesis (Biron 1999). In vitro data show that John Cunningham virus (JCV) infection induces IFN-α and IFN-β that results in significant inhibition of JCV gene and protein expression (Verma et al. 2006; Co et al. 2007).

Therefore, we hypothesized that the prevalence of JCV DNA in blood and urine would be suppressed in patients receiving IFN-β1a (Avonex or Rebif) or IFN-β1b (Betaseron). On the other hand, we anticipated that glatiramir acetate (GA, Copaxone) would have no effect on the prevalence of virus and that immunosuppressive regimens and natalizumab would increase viral copy numbers.

Subjects

The study was conducted at the Multiple Sclerosis Center of the University of Kentucky in Lexington, Kentucky. This study was approved by the Institutional Review Board of the University of the Kentucky College of Medicine, and patients provided informed consent to participate. The study was comprised of two components, a cross-sectional study and a longitudinal study. Two hundred thirty-nine patients were enrolled in the cross-sectional study. Peripheral blood mononuclear cells (PBMCs) and urine were obtained from 239 MS patients [180 women, 59 men; mean age 45.2 years (range 18–70)] in the cross-sectional study. MS classification for the cross-sectional group included 172 with relapsing–remitting MS (RRMS), 2 with benign MS, 44 with secondarily progressive MS (SPMS), 15 with primary progressive MS (PPMS), 4 with relapsing progressive MS (RPMS), and 2 with neuromyelitis optica. Forty of these patients were on no disease-modifying drugs (DMDs), and 199 were treated with a variety of MS regimens, IFN-βs (121), GA (71), and other MS regimens, e.g., natalizumab, mitoxantrone, and others (7; Table 1). All patients had received a stable DMD regimen for ≥6 months (ranges 7–194 months). The mean and median times on the current DMD were 73 and 60 months, respectively. Eighty-six (36 %) of 239 patients had received other MS therapies before the initiation of the current DMD. The JCV antibody seroprevalence rate in a subset of 64 individuals from our MS clinic was 60.9 %.

The longitudinal study was comprised of 37 individuals who were treatment naïve and newly diagnosed with MS [29 women, 8 men; mean age 40.2 (range 21–63)] (Table 2). MS classification revealed 32 RRMS, 2 RPMS, and 3 PPMS. Blood and urine for PCR for JCV were obtained at the time of diagnosis and 6 months after treatment initiation. Three patients in the longitudinal group remained treatment naïve.

Methods

Blood and urine were obtained on all patients at the time of study entry. For treatment-naïve patients enrolled in the longitudinal study, blood and urine were collected again at the time of their 6-month routine follow-up visit to the clinic.

Total DNA was purified from enriched buffy coat, derived from the equivalent of 1.5 ml whole blood, using the QIAGEN Blood kit and eluted in 200 μl buffer AE. Buffy coat was selected as we had previously demonstrated that plasma and sera were no more sensitive than buffy coat for demonstrating JCV viremia (Berger et al. 2006). Total DNA was purified from 1 ml urine using the QIAamp Viral RNA Mini kit and eluted in 70 μl water. Ten microlitres of purified DNA was subjected to real-time quantitative PCR using primers and probes specific for JCV detection, namely the large tumor (T) antigen as described by MacKenzie et al. (2003). The JCV probe was labeled at the 3′ end with the quencher fluorochrome, 6-carboxytetramethyl-rhodamine (TAMRA; PE Applied Biosystems). The 5′ end of the probe was labeled with the reporter fluorochrome, 6-carboxyfluorescein (6-FAM). Real-time PCR was performed on an ABI Prism 7700 Sequence Detection system (PE Applied Biosystems). Cycling parameters were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Each PCR run contained negative controls, including reaction mixtures without DNA template and several specimens that were known to contain no JCV DNA. Positive controls consisted of a 10-fold dilution series (1×10^0 to 1×10^6 genome equivalents per reaction) of cloned JCV (MAD1 plasmid) sequences kindly provided by E.O. Major (NINDS) who has performed quality control studies of our assay. Each specimen was analyzed in duplicate. Results were scored positive if either reaction yielded a threshold cycle value (Ct) above the limit of detection for the standards. We reliably detect 10 copies/μl of purified template which is the equivalent of 35 copies/mL of body fluid. For statistical analysis, chi-square test was used to determine the effect of disease-modifying drugs on JC viruria. Similarly, chi-square tests were used to determine differences in the presence or absence of JC viruria between genders. Age was compared between groups using *t* test. Since the measurements were not normally distributed, the nonparametric Mann–Whitney test was performed to compare JCV in urine (limited to positives) between genders and between medication and no medication. The logistic model

Table 1 Demographics of the cross-sectional study

Current medication	Absolute number	Average age (years)	Age range (years)	Female	Male	Average duration of MS (years)
Avonex	25	49	26–70	23	2	7
Betaseron	55	46	19–67	36	19	6.2
Copaxone	71	43	22–64	49	22	7
Novantrone	1	58	58	1	0	3
Rebif	41	45	18–66	33	8	4.1
Tysabri	4	43	20–61	3	1	9
None	40	46	18–69	33	7	9
Other	2	44	38–50	2	0	10

was used to determine whether medication is a significant predictor of JC viruria positivity after adjusting for demographic factors. In the longitudinal study, percentages of JCV positive before and after medication were compared using McNemar's test, while JCV in urine was compared between the two using Wilcoxon signed rank sum test.

Results

JCV was not detected in the PBMCs of any patients on study entry. Viral DNA was detected in two patients in the 6-month follow-up blood sample, one following IFN-β1b subcutaneously and the other following the initiation of natalizumab. The small number precluded meaningful comment. JCV was detected in the urines of 112 (46.8 %) of the 239 urines collected in the cross-sectional arm of this study (Table 3) and in 19 (51.4 %) of 37 urine specimens collected in the longitudinal study (Table 4). There was no effect on either prevalence or copy numbers of JCV DNA by any of the standard platform therapies (GA, IFN-β1a intramuscular, IFN-β1a subcutaneously, or IFN-β1b subcutaneously). The infrequency of other therapies did not permit meaningful statistical analysis. The mean age of those with JC viruria (46.8 years) was marginally higher than the group as a whole (45.2 years). JCV DNA was more commonly detected in the urine of men (60.2 %) when compared to women (46.4 %; $p=0.03$). There was no statistically significant effect of DMDs on urinary JCV prevalence and/or viral copy numbers when age or gender was considered as a co-factor.

Of the 37 subjects evaluated longitudinally, urine remained free of detectable levels of JCV DNA in 14 subjects. JCV DNA was detected in one of two visits for 13 subjects, with 9 converting to JCV positive and 4 to JCV DNA negative. In all but one of these cases, JCV viral loads were very low, ranging from 41 to 1,006 genome copies/mL, whereas one subject had a viral load of 1.903×10^7 copies/mL at the second visit only. JCV DNA was detected at both visits in urine obtained from 10 subjects. The JCV load increased in six subjects (four IFN-β1b and two GA) and declined four patients (two IFN-β1b and two IFN-β1a). Of five patients with high viral loads at the first visit, viral load dropped >2 orders of magnitude on IFN-β1a, increased 3.7 fold with IFN-β1b in one patient, and increased 1.3- and 4.0-fold in two patients on GA. When IFN-βs were analyzed as a group, there was no statistically significant effect on JCV viruria.

Discussion

While JCV present in the PBMCs of only 2 (<1 %) of 276 patients, it was detected in 131 (47.5 %) of 276 urine

Table 2 Demographics of the longitudinal study

Current medication	Absolute number	Average age (years)	Age range (years)	Female	Male
Betaseron	15	42.1	22–63	13	2
Copaxone	6	39	25–49	5	1
Rebif	9	41.7	25–56	6	3
Tysabri	3	26	21–29	3	0
None	3	42.3	31–48	2	1
Alemtuzumab	1	43	43	0	1

Table 3 JC viruria in relationship to the MS treatment regimen

Current medication	Absolute number	Absolute number of JCV+	Percentage positive	Average number of JCV (copies/mL)	Range of JCV (copies/mL)
Avonex	25	8	32	3.594×10^{7}	$0–4.972\times10^{8}$
Betaseron	55	29	52.70	1.106×10^{8}	$0–3.552\times10^{9}$
Copaxone	71	36	50.70	2.352×10^{7}	$0–8.077\times10^{8}$
Novantrone	1	0	0	0	0
Rebif	41	19	46.34	1.801×10^{7}	$0–5.049\times10^{8}$
Tysabri	4	1	25	84	0–337
None	40	19	47.50	2.534×10^{7}	$0–9.161\times10^{8}$
Other	2	0	0	0	0
All interferon-βs	121	56	46.3	6.850×10^{7}	2.182×10^{8}

samples (239 samples collected from the of entry into the cross-sectional study and 37 samples collected at the time of return visit 6 months after DMD initiation in the longitudinal population). These observations are consistent with prior studies that indicate that JCV DNA is rarely detected in the blood of otherwise immunocompetent persons, but is present in one third or more of urines of healthy individuals (Pagani 2003; Polo et al. 2004; Berger et al. 2006). Urine JCV DNA was more commonly detected in men than women ($p<0.001$), and the mean age of JCV patients was higher (46.8 years) than the group as a whole 45.2 years. Neither finding is unanticipated. An increase frequency of JCV antibody seropositivity in men has been previously reported (Gorelik et al. 2010) but has not been universally observed (Egli et al. 2009). Similarly, the effect of age on urine JC viruria is not unexpected. JCV antibody seropositivity increases with age (Egli et al. 2009; Gorelik et al. 2010), and there is a greater likelihood of detecting the virus in the urines of elderly JCV antibody seropositive individuals (Chang et al. 2002). There was no effect of MS classification (RRMS, PPMS, SPMS) on JCV DNA prevalence in the urine. The small number of patients receiving therapies other than the platform drugs in this study precluded meaningful analysis of their effect on JCV prevalence or copy numbers.

This study failed to show an effect of either the IFN-βs, individually or as a group or GA on the prevalence or copy numbers of JCV in PBMCs or urine of treated MS patients. Neither the percentage of patients with detectable JCV in their urine nor the quantity of JCV DNA was affected by either class of DMDs in either the cross-sectional or longitudinal study. Another study similarly compared IFN-β-treated patients with RRMS to untreated patients (Alvarez-Lafuente et al. 2007). In that study, 6.8 % (5 out of 73) of patients had JCV detected in their PBMCs; however, concordant with our observations, there was no difference in JCV viral load in PBMCs between the IFN-β-treated and untreated MS patients (Alvarez-Lafuente et al. 2007). The results of these studies contrast with one that compared 59 IFN-β-treated RRMS patients to 39 untreated RRMS patients and 98 healthy controls. These investigators detected JCV

Table 4 JC viruria in the longitudinal study

Medication initiated	Number of patients	Number and percentage with JCV positivity at visit A	Number and percentage with JCV positivity at visit B	Average JCV (copies/mL) at visit A	Average JCV (copies/mL) at visit B	Difference in average JCV (copies/mL) from visit A to visit B	Range of JCV copies (visit A)	Range of JCV copies (visit B)
Betaseron	15	7 (46.7 %)	10 (66.6 %)	2.671×10^{5}	1.237×10^{6}	9.695×10^{5}	$62–5.401\times10^{4}$	$0–1.753\times10^{7}$
Copaxone	6	1 (16.7 %)	2 (33.3 %)	8.148×10^{7}	3.402×10^{8}	2.587×10^{8}	$357–3.345\times10^{8}$	$0–1.686\times10^{9}$
Rebif	9	3 (33.3 %)	4 (44.4 %)	3.170×10^{7}	6.960×10^{4}	-3.162×10^{7}	$212–2.566\times10^{8}$	$0–5.6.12\times10^{5}$
Tysabri	3	0	2 (67 %)	0	6.309×10^{6}	6.309×10^{6}	0	$0–1.892\times10^{7}$
None	3	1 (33.3 %)	1 (33.3 %)	380	333	−48	1,142	0–1000
Alemtuzumab	1	0	0	0	0	0	0	0

The table shows the absolute viral copy numbers at the initial visit (A) and 6 months after initiation of disease-modifying therapy. The difference in copy numbers for each group between the two visits is also displayed. Three patients remained untreated

DNA in the peripheral blood of 13.6 % of IFN-β-treated MS patients versus 46.1 % of untreated MS and 28.6 % of healthy controls (Delbue et al. 2007). Interestingly, PML has been seen to progress despite the IFN-α (Berger et al. 1992), another type 1 interferon, and IFN-β (Nath et al. 2006) treatment consistent with the lack of an effect on JCV expression observed in our study and others.

The first two patients with natalizumab-associated PML had been treated with intramuscular IFN-β (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005). Until additional cases were observed in the absence of the co-administration of IFN-β, there was a concern that combination therapy contributed to the development of PML. With all but 2 cases of natalizumab-associated PML occurring without the co-administration of other DMDs coupled with the observation that there has been no reported effect of either IFN-β or GA on the occurrence of PML, this study suggests that IFN-β or GA could probably be safely used in conjunction with natalizumab or other drugs that may increase the risk of PML.

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