Washed Cycad Flour Contains β-N-methylamino-L-Alanine and May Explain Parkinsonism Symptoms

Sandra Anne Banack, PhD,[1] Peter B. Nunn, PhD,[2] Ran Cheng, BS,[3] and Walter G. Bradley, DM, FRCP[4]

The study reported by Dr. Shen and the group from the University of Maryland[1] and their follow-up study of sleep alterations in their new model of parkinsonism produced by feeding cycad flour to rats[2] are very interesting. Shen et al mention β-N-methylamino-L-alanine (BMAA) as one of the incriminated neurotoxins in cycad seeds and review the work of the Vancouver group on plant sterols in washed cycad flour. In discussion of the factor(s) responsible for the neurotoxicity in this new rat model, it is important to remember that washing cycad flour removes only free BMAA, and that 7 to 30× as much BMAA remains within the protein fraction of washed cycad flour.[3] We found that cycad flour contained from 28 to 169μg/g of protein-bound BMAA, depending on the washing procedure.[3,4] Duncan et al[5] found that, despite washing, cycad flour prepared by Chamorros and sold at village markets contained up to 152μg/g of free BMAA, suggesting that the protein-bound BMAA fraction in his samples might have been even higher than we found.

Potential Conflicts of Interest

W.G.B. has worked as a consultant for information companies regarding amyotrophic lateral sclerosis and therapeutic trials.

[1]Institute for Ethnomedicine, Jackson, WY, [2]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK, [3]Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, and [4]Department of Neurology, Miller School of Medicine, University of Miami, FL

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Reply

Kimberly A. McDowell, BS,[1] Wei-Bin Shen, PhD,[2] Aubrey A. Siebert, BS,[1] Sarah M. Clark, BS,[2] H.A. Jinnah, MD, PhD,[4] Carole Sztalryd, PhD,[5] Paul S. Fishman, MD, PhD,[3,6] Christopher A. Shaw, PhD,[7,8,9] M. Samir Jafri, PhD,[1,3,6] and Paul J. Yarowsky, PhD[1,2,3,10]

β-Methylamino-L-alanine (BMAA) has been proposed as a toxic agent damaging several neuronal types in amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS-PDC). BMAA has been shown to cause neurodegeneration in vitro.[1] We agree that BMAA could be an important component in the unwashed cycad flour and thank the authors for the suggestion. In their Letter, Banack and colleagues propose that BMAA-induced toxicity involves the incorporation and/or biomagnification of this nonprotein amino acid into a bound form or "toxic reservoir" that slowly releases the compound eventually producing neurological effects. However, work by Cruz-Aguado and colleagues[2] examined oral administration of BMAA alone (28mg/kg/day) in mice, and found no signs of either an acute or a chronic neurodegeneration. In other in vivo studies injecting BMAA (intravenous, subcutaneous, or intraventricular), the behavioral phenotype observed in those studies was not a progressive and irreversible neurodegeneration as we have observed when feeding rats washed cycad flour.[3] In addition, although Murch and colleagues[4] report cortical levels of BMAA in patients with PDC or Alzheimer's disease of 3–10μg/g of free BMAA and 149–1190μg/g of bound BMAA, an independent study of analysis of BMAA in tissue (a multidimensional chromatographic method and a stable isotope dilution technique) detected only trace amounts of free BMAA in the cerebrum of mice fed BMAA for 1 month at a dose of 28mg/kg of body weight (BW)/day and no bound BMAA was detected. If we make the assumption that the protein-bound form of BMAA is in fact at concentrations of 169μg/g of cycad flour in the cerebrum of cycad-fed mice, previous in vivo studies do not show a correlation between the amounts of BMAA ingested and ALS/PDC-like phenotypes in mice. Using this 169μg/g concentration for bound BMAA, our recently published study feeding rats 1.25g cycad/day is equivalent to an ingested dose of 0.53mg/kg BW/day.[3] Also, in contrast to Murch and colleagues,[4] BMAA was not detected by Snyder and colleagues[5,6] in human brain cerebral cortical extracts from PDC patients or controls from Guam or from the Seattle area with Alzheimer's disease. This suggests that the BMAA is an unlikely toxin candidate for our model of cycad flour–induced parkinsonism.

[1]Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD
[2]Department of Pharmacology and Experimental Therapeutics, and
[3]Research Service, Maryland VA Health Care System, Baltimore, MD

[4]*Department of Neurology, Emory University, Atlanta, GA*
[5]*Department of Gerontology, and*
[6]*Department of Neurology, University of Maryland School of Medicine, Baltimore, MD*
[7]*Department of Ophthalmology and Visual Sciences,*
[8]*Department of Experimental Medicine, and*
[9]*Neuroscience Programme, University of British Columbia, Vancouver, BC, Canada*
[10]*Program in Toxicology, University of Maryland School of Medicine, Baltimore, MD*

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Stalevo Reduction in Dyskinesia Evaluation in Parkinson's Disease Results Were Expected from a Pharmacokinetic Viewpoint

Dag Nyholm, MD, PhD, Håkan Askmark, MD, PhD, and Sten-Magnus Aquilonius, MD, PhD

The recently reported results from the Stalevo Reduction in Dyskinesia Evaluation in Parkinson's Disease (STRIDE-PD) study were described as surprising because dyskinesias were increased rather than decreased.[1] The study design was based on animal data and a belief that "the addition of entacapone to L-dopa . . . provides more continuous L-dopa availability."[1] If this feature of cathecol-O-methyltransferase (COMT) inhibitors were true, the concept of continuous dopaminergic stimulation (CDS) must be questioned considering the new results. However, addition of a COMT inhibitor, either entacapone or tolcapone, does not produce stable L-dopa concentrations in plasma, even if the brand name Stalevo may suggest that it does. Even if trough concentrations of L-dopa increase somewhat more than peak concentrations, the fluctuating pattern related to each intake of oral L-dopa is not altered by COMT inhibitors. This has been demonstrated previously,[2] as recently as May 2009 by Kuoppamaki and colleagues,[3] who found that "no significant differences were generally seen in variability (Cmax–Cmin) of levodopa concentrations during the day between [L-dopa/carbidopa/entacapone] LCE and [L-dopa/carbidopa] LC." Two of the authors of that article were thanked in the acknowledgment of the STRIDE-PD article, but their work was not cited. Instead, Reference 30 in Stocchi and colleagues[1] was used to support the statement that entacapone "provides more continuous L-dopa availability." Reference 30 is a review article, containing a figure of pharmacokinetic profiles of L-dopa with and without entacapone. The figure legend states that entacapone "provides a pharmacokinetic profile that strikingly resembles that seen with a levodopa infusion." The figure is taken from an earlier article by Drs. Olanow and Stocchi,[4] published in a Novartis/Orion-sponsored supplement of *Neurology*. The original figure legend states that the pharmacokinetic profiles were taken from 1 single patient. Plasma samples were collected hourly, which is highly unreliable in studies of L-dopa. Half-hourly measurements are available in the literature, both with oral LCE delivery and intestinal infusion of LC.[3,5] There is no evidence that entacapone provides L-dopa profiles that resemble those seen with infusion.

Thus, the STRIDE-PD study brought no new insight into the CDS concept because COMT inhibitors cannot significantly alter the fluctuating plasma profiles of orally administered L-dopa. Therefore, the results of the STRIDE-PD study are not surprising given the peer-reviewed literature on L-dopa pharmacokinetics-pharmacodynamics in PD as a background.

Potential Conflicts of Interest

D.N. serves as a consultant to Abbott Products, the manufacturer of levodopa/carbidopa intestinal gel (Duodopa®), and to Sensidose AB, who are developing an electronic dose dispenser for levodopa/carbidopa microtablets. He has also received lecture fees from Orion Pharma, the manufacturer of entacapone and levodopa/carbidopa/entacapone, and remuneration from H. Lundbeck. H.A. is a member of H. Lundbeck's advisory board for neurology in Sweden. S.-M.A. is co-founder of Neopharma Production AB and Sensidose AB. None of the companies are involved in the present letter.

Department of Neuroscience, Neurology, Uppsala University, Uppsala, Sweden

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Reply

C. Warren Olanow, MD[1] and Fabrizio Stocchi, MD[2]

We have previously hypothesized that L-dopa–induced motor complications result from intermittent or pulsatile stimulation of denervated striatal dopamine receptors due to the short half-life of the drug.[1] The concept of continuous dopamine stimulation suggests that treatment with a continuous or long-acting formulation of L-dopa might reduce the risk of motor complications. We have previously demonstrated that continuous infusion of L-dopa reduces both off time and dyskinesia in comparison to intermittent administration of a standard formulation of L-dopa.[2] These benefits were observed although pharmacokinetic (PK) studies demonstrated that plasma L-dopa levels were not maintained at a continuous level, and indeed plasma levodopa AUC was greater than with oral administration. Rather, we proposed that the benefits obtained were related to avoiding the low plasma L-dopa trough levels that might translate into low striatal dopamine levels and periods in which striatal dopamine receptors are not activated, thus resulting in discontinuous or pulsatile stimulation. Although this proof of concept study supported the concept of continuous dopaminergic stimulation (CDS) as a treatment for Parkinson disease (PD), continuous infusion is an impractical therapeutic alternative especially for patients with early stage disease. In the STRIDE-PD study, we combined L-dopa with a catechol-O-methyltransferase (COMT) inhibitor to extend the elimination half-life of the drug and thereby tried to obtain CDS. We have performed PK studies demonstrating that L-dopa 100mg combined with 200mg of the COMT inhibitor entacapone administered 5× daily at 3-hour intervals prevents the low trough levels seen with regular L-dopa (Fig) and provides a PK profile resembling what is obtained with continuous intraintestinal L-dopa infusion. PK studies published by Kuoppamaki et al showed similar results[3] and were cited in our original manuscript, but unfortunately were removed in error during the editing process. For this we apologize.

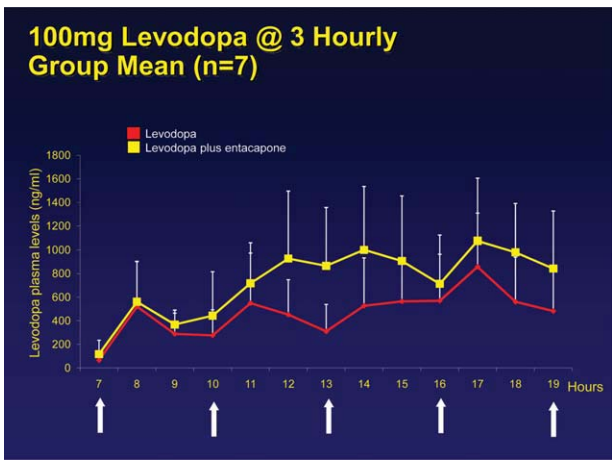

FIGURE: Mean L-dopa plasma concentrations in 7 Parkinson disease patients receiving L-dopa administered alone or in combination with a catechol-O-methyltransferase (COMT) inhibitor at 3-hour intervals. Note that low trough levels associated with regular L-dopa are partially avoided when L-dopa is combined with a COMT inhibitor. [Color figure can be viewed in the online issue, which is available at annalsofneurology.org.]

The STRIDE-PD study study failed to show the anticipated benefit.[4] As we discussed in the paper, we believe this is likely because administration of L-dopa plus a COMT inhibitor four× daily failed to achieve CDS. The higher frequency of dyskinesia in the L-dopa/COMT inhibitor group probably reflects the tendency for a greater dopaminergic load to induce a higher frequency of dyskinesia. This might not have been the case had CDS been achieved, as continuous L-dopa infusion provided reduced off time and reduced dyskinesias in our pilot study, although subjects had a higher L-dopa load and a greater levodopa plasma AUC than those in the L-dopa alone group.[2] In methylphenyltetrahydropyridine-treated monkeys, administering L-dopa with a COMT inhibitor reduced dyskinesia when CDS was achieved, but increased dyskinesia when given less frequently (presumably not achieving CDS).[5] We think that Aquilonius et al are premature in assuming that COMT inhibitors used as an adjunct to L-dopa cannot provide CDS, as the correct dosing paradigm has probably not been tested. We believe that administering L-dopa with a COMT inhibitor or in a long-acting oral or transdermal formulation remains an important area for further investigation in an attempt to obtain the benefits of L-dopa with reduced motor complications.

Potential Conflicts of Interest

Both Dr Olanow and Dr Stocchi have served as consultants for both Novartis and Orion. Non-relevant conflicts are the same as those listed in the main article.

[1]*Department of Neurology, Mount Sinai School of Medicine New York, NY*
[2]*Department of Neurology, Institute of Neurology (FS, CWO), IRCCS San Raffaele Pisana, Rome, Italy; INSERM U455*

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A Novel Hypothesized Clinical Implication of Zonisamide for Autism

Ahmad Ghanizadeh, MD

In an interesting recently published study, Asanuma et al reported that zonisamide, which is used as an antiepileptic medication and a novel antiparkinsonian drug, significantly increases glutathione levels in astroglial cells.[1] Zonisamide enhances cystine/glutamate exchange and increases influx of cystine. Cystine is an important substrate for glutathione synthesis, and glutathione is an antioxidant. Herein, I would like to discuss another possible clinical and research implication of their findings.

Autism is neurodevelopmental disorder, the prevalence of which is rising. Its etiology is not exactly known, and there is no curative treatment. Methionine, cystine, and glutathione concentration in children with autism were found to be lower than in a control group,[2] whereas a higher concentration of oxidized glutathione was reported in children with autism.[2] This lower methionine concentration is due to reduction in methionine synthase activity.[2] A consequence of decreased methionine cycle turnover is decrease of cystine and glutathione synthesis.[2] Glutathione is made from cystine, glutamate, and glycine. Cystine has an important role in the synthesis of glutathione, because its concentration is lower than that of glycine and glutamate. Therefore, cystine has a limiting role for glutathione synthesis. One of the sources of cystine is direct import from plasma.[3] Another source of cystine is via methionine cycle.[3] Low cystine concentration decreases glutathione synthesis.[4] The role of glutathione in antioxidant effect is clear in many disorders, such as parkinsonism and autism. Low glutathione increases vulnerability to oxidative stress. Increased oxidative stress and decreased methylation capacity and glutathione concentration contribute to autism clinical manifestation.[2] Demand for cystine, methionine, and glutathione is increased during chronic oxidative stress[4] such as autism. Lower concentration of methionine, cystine, and glutathione is suggested as a metabolic biomarker of autism.[2] Therefore, there is a possibility that providing more cystine increases methionine cycle and glutathione. This can improve antioxidant activity.

Given that methionine, cystine, and glutathione concentration in children with autism is low, oxidative stress in autism is increased, and demand for cystine, methionine, and glutathione during chronic oxidative stress is high.

Regarding zonisamide, which is a safe medication in young children[5] that increases glutathione by increasing the influx of cystine,[1] another implication of their findings[1] is the need to conduct studies for the assessment of the possible efficacy of zonisamide administration for the management of autism. In conclusion, even if zonisamide may impact on other neurotransmitters, and the mechanism by which it works is still not completely understood, the present hypothesis suggests that conducting clinical trials on this matter may be worthwhile.

Department of Child and Adolescent Psychiatry, Shiraz University of Medical sciences, Shiraz, Iran

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Reply

Masato Asanuma, MD, PhD and Ikuko Miyazaki, PhD

We thank Dr Ghanizadeh for his interesting hypothesis related to the glutathione (GSH)-increasing effect of zonisamide (ZNS) in our article.[1] We had not considered a possible clinical application of ZNS for autism until we received the comment. A growing number of reports have demonstrated the involvement of oxidative stress in the pathophysiological alteration of autism spectrum disorders,[2] especially reduction GSH, GSH/oxidized GSH (GSSG) ratio, and cysteine in both cytosol and mitochondria of lymphoblasts in autism.[3] Furthermore, administration of antioxidant, high-dose ascorbic acid or carnosine improved autistic behavior in double-blind, controlled studies of children with autism.[4,5] These findings may allow us to propose the possible clinical implication for autism of ZNS that highly distributes to the central nervous system and increases GSH levels in the brain. However, it is unclear how reduction of GSH contributes or relates to the behavioral symptoms in autism, and there is no evidence regarding efficacy of high-dose GSH or its precursor as treatment in autism. Therefore, further clinical studies of GSH supplementation are needed to understand the possible application of ZNS for autism.

ZNS possesses multiple pharmacological effects[6]: inhibition of Na channels or Ca channels, increasing glutamate transporter GLT-1 and tyrosine hydroxylase expression, enhancing dopamine release, antitremor effects, antiapoptotic effects, antioxidative effects, and neuroprotective effects in several parkinsonian models, including the results of our study. We demonstrated not only the GSH-increasing effects of ZNS but also its astrocyte-proliferating effects.[1] Among the various pharmacological properties of ZNS, some of them (eg, activation of GLT-1, antioxidative effects, and neuroprotective effects) may be based on its astrocyte-increasing effects. Transient reduction of GLT-1 on astrocytes and dysfunction of astrocytes in the hippocampus contributes to delayed hippocampal neuronal death after transient ischemia, which is protected by up-regulation of GLT-1 using ceftriaxone.[7] Neurodegeneration of motor neurons in Cu/Zn superoxide dismutase mutant transgenic mouse model of amyotrophic lateral sclerosis (ALS) was prevented by overexpression of antioxidant transcriptional master protein Nrf2 and GSH secretion in astrocytes,[8] and also protected by transplantation of astrocytes, in part via its GLT-1 function.[9] Thus, these recent reports regarding astrocyte dysfunction and effects of the replacement in ischemic neuronal damage and the ALS model

imply possible clinical applications of ZNS, which enhances the astroglial antioxidative and neuroprotective function, for these neurodegenerative disorders.

Department of Brain Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

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Neuropathy in Idiopathic Parkinson Disease: An Iatrogenic Problem?

Maria Nolano, MD,[1] Vincenzo Provitera, MD,[1] Bernardo Lanzillo, MD,[1] and Lucio Santoro, MD[2]

We read with interest the article by Toth et al reporting a high occurrence (55%) of neuropathy in idiopathic Parkinson disease (IPD).[1] They found a strong association, without demonstrating a causative effect, between L-dopa exposure, methylmalonic acid level, and neuropathy severity.

Previously we reported, in 18 IPD patients, epidermal nerve fiber (ENF), Meissner corpuscle (MC), and sensory loss more evident on the more affected side,[2] without correlation with age, disease duration, and apparently drug treatment (4 patients were untreated). MC loss correlated instead with disease severity.

These findings suggest that the neuropathic involvement occurs early in IPD, representing 1 of the neuropathological aspects of a multisystem neurodegenerative disease.[2,3] However, inspired by Toth's work, we calculated the cumulative L-dopa intake for each of our IPD patients and studied 3 more untreated subjects. We found that L-dopa exposure did not correlate with ENF density in thigh ($p = 0.36$), leg ($p = 0.25$), and fingertip ($p = 0.37$), whereas it correlated ($p < 0.05$) with MC density (Fig A), in agreement with the association between L-dopa intake and nerve conduction velocity (NCV) found by Toth. In fact, both MC count and NCV explore, in different ways, large fibers. However, in our patients, L-dopa intake correlated also ($p < 0.02$) with disease severity (see Fig B), which in turn correlated ($p < 0.05$) with MC loss (see Fig C). The same correlations between L-dopa exposure, IPD severity, and neuropathy were reported by Toth, leaving unclear the role of therapy and disease severity in the development of large fiber neuropathy in IPD.

Examining our data from treated and untreated patients separately, we observed a loss of ENF in both groups (see Fig G, I vs E), whereas a loss of MCs was present only in the treated patients (see Fig , H vs D). In untreated patients, MCs showed, however, evident morphological anomalies (see Fig F vs D). ENF loss in IPD appears, then, unrelated to drug treatment. Conversely, we could not determine the exact role of L-dopa treatment and disease severity in the degeneration of large fiber endings.

Finally, Toth's electrophysiological findings show a discrepancy with ours (abnormality = 55% vs 0%) that resolves considering the different Unified Parkinson Disease Rating Scale scores (42.1 ± 27.9 vs 26.6 ± 10.2) and L-dopa exposure (1.72 ± 1.14 kg vs 1.03 ± 1.39 kg) of the 2 populations. Our patients are more similar to the subgroup without neuropathy studied by Toth, which, however, could have shown morphological abnormalities using skin biopsy.

Therefore, a larger study including both electrophysiological and morphological methods should be performed to evaluate the neurotoxicity of L-dopa and the possible protective effect of cobalamin supplementation.

Potential Conflicts of Interest

Nothing to report.

[1] *"Salvatore Maugeri" Foundation, Institute for Scientific Research and Care, Medical Center of Telese Terme, Telese Terme, Benevota, Italy*
[2] *Department of Neurological Sciences, University of Naples "Federico II", Naples, Italy*

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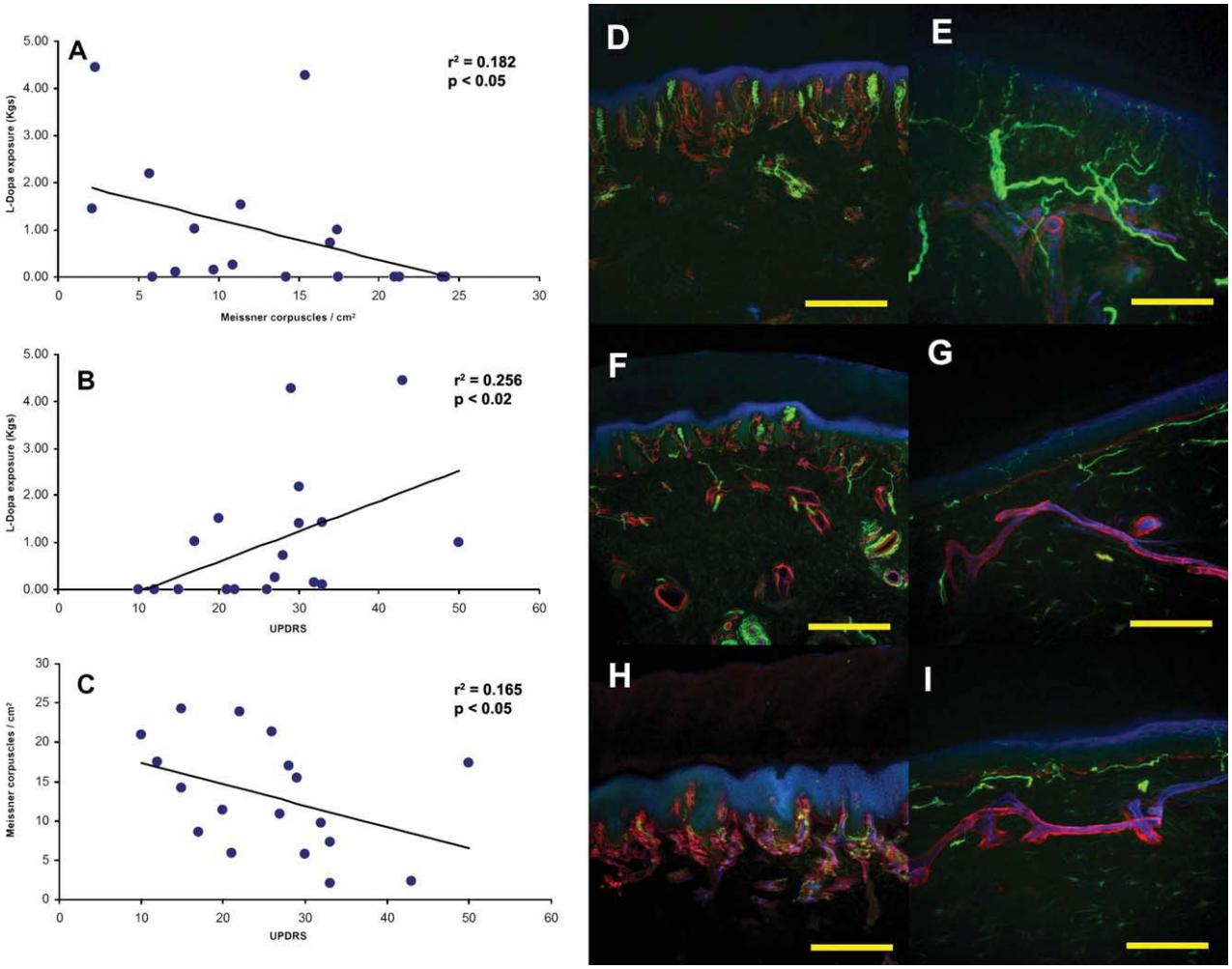

FIGURE: Scatterplots show correlations in idiopathic Parkinson disease (IPD) patients: (A) between Meissner corpuscle (MC) density and the total exposure to L-dopa, (B) between L-dopa exposure and disease severity as expressed by the Unified Parkinson Disease Rating Scale (UPDRS) score, and (C) between UPDRS score and MC density. (D–I) Digital confocal images show glabrous (D, F, and H) and hairy (E, G, and I) skin from a normal subject (D, E) and an untreated (F, G) and a treated (H, I) IPD patient. In green, nerve structures are marked with protein gene product 9.5; in red, vessels and basement membrane are marked with collagen IV; in blue, endothelia are marked with *Ulex europaeus*. Biopsies from glabrous skin are taken from fingertip; biopsies from hairy skin are taken from distal leg. MC density is not significantly reduced in untreated IPD patients compared to controls, although morphological abnormalities of mechanoreceptors are already present. In treated IPD patients, the MC degenerative process is fully manifested. In IPD patients, a reduction of epidermal nerve fiber density occurs regardless of L-dopa therapy. Scale bar = 400μm in D, F, and H, and 100μm in E, G, and I.

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Reply

Cory Toth, MD, Douglas Zochodne, MD, and Oksana Suchowersky, MD

We thank Dr Nolano and colleagues for their comments regarding our article examining the prevalence of peripheral neuropathy in patients with idiopathic Parkinson's disease (IPD).[1] They have examined cutaneous innervation in patients with IPD, determining that loss of epidermal nerve fibers (ENF) and Meissner corpuscles (MC) occurs in IPD patients, with MC loss associated with IPD severity.[2] In our work, we postulated that the pathophysiological cause of peripheral neuropathy in IPD patients may be indirectly related to levodopa accumulative usage. Nolano and colleagues performed a post hoc analysis on their own data, and determined that MC loss correlated with levodopa intake. As we had stated previously, the association of levodopa usage and fasting methylmalonic acid with peripheral neuropathy has not been shown to be causative, as also explained by Nolano and colleagues. Interestingly, only IPD patients receiving levodopa therapy had depressed MC levels.

There are limitations in the data presented by Nolano and colleagues—only a small number (n = 18) of patients were assessed and we are not privy to their levodopa intake, fasting methylmalonic acid levels, clinical severity, and potential use of vitamin therapy. These confounders make definite interpretation of their data difficult. Likewise, comparisons to data obtained with our IPD patient and control subject data are not possible. Finally, they report that IPD patients (n = 3) without a history of levodopa use also had reductions in epidermal nerve fiber density—it is important to note that other causes for peripheral neuropathy may occur in populations of patients with IPD, as we previously discussed.[3] We agree with Nolano and colleagues that further studies are required to understand the development of peripheral neuropathy in IPD patients and the role of cobalamin deficiency; such studies may indeed contain epidermal nerve fiber density measurements.

Hotchkiss Brain Institute, University of Calgary, Calgary Alberta, Canada

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Assay Design and Sample Collection Can Affect Anti-John Cunningham Virus Antibody Detection

Susan E. Goelz, PhD,[1] Leonid Gorelik, PhD,[1,2] and Meena Subramanyam, PhD[3]

Two articles published in the September issue of *Annals of Neurology* describe the detection of anti-John Cunningham virus (JCV) antibodies in multiple sclerosis (MS) and progressive multifocal leukoencephalopathy (PML) patients.[1,2] We would like to point out an area of discrepancy between the reports.

In our study, 100% of pre-PML blood samples (n = 17) were found to be seropositive,[2] whereas 3 of 25 post-PML samples in the Ryschkewitsch et al study were negative (n = 1) or borderline positive (n = 2) for anti-JCV antibodies.[1] The Ryschkewitsch et al results were interpreted in an accompanying editorial[3] as demonstrating a 12% false-negative rate for PML patients, implying that anti-JCV antibody serology may not be an adequately sensitive method for detecting JCV presence or for PML risk stratification. However, it is essential to note that all 3 of these post-PML samples that tested negative or borderline positive in the Ryschkewitsch assay were collected during plasma exchange (Table), which is used to remove natalizumab from the circulation. Because plasma exchange also removes other antibodies from the circulation, the concentration of anti-JCV antibodies would also be reduced. Importantly, despite removal of antibodies by plasma exchange, duplicates of these same samples were found to be positive in our assay (see Table), thus confirming the high sensitivity of our assay for JCV-specific antibodies.

This discrepancy is likely due to the difference in the assay designs. Ryschkewitsch et al[1] used a 1-step solid-phase enzyme-linked immunosorbent assay (ELISA) that identified 65% of MS patients as being seropositive. Generally, when using this type of assay, as sensitivity is increased by lowering the cutoff point, the number of false-positive samples increases due to low-level nonspecific antibody binding. Similar to Ryschkewitsch et al, the first step of our assay (also a solid-phase ELISA) identified 70% of patient samples as above the cutoff point. However, the unique feature of our assay is the second confirmation step, which was designed to minimize samples with nonspecific binding in the solid-phase

TABLE: Anti-JC-virus Antibody Status and Timing of Sample Collection Relative to Plasma Exchange[a] in 3 PML Cases

Patients as Designated in Ryschkewitsch et al[1]	1-Step Assay, Ryschkewitsch et al[1]	2-Step (ELISA + confirmation test) Assay, Gorelik et al[2]	Sample Collection Date	PLEX Dates
PML Pt 2 (#4)	Borderline positive	Positive[b]	Dec 5, 2008	[b]IA, 4 cycles: Dec 3, Dec 5, Dec 8, Dec 10
PML Pt 16 (#14)	Negative	Positive[b]	Jul 29, 2009	[b]PLEX, 3 cycles: Jul 24, Jul 27, Jul 29
PML Pt 18 (#21)	Borderline positive	Positive[b]	Oct 7, 2009	[b]PLEX, 5 cycles: Oct 1–8

[a]MS patients with PML undergo plasma exchange to remove natalizumab from the circulation.
[b]Unpublished (subject of a separate article).
PML = progressive multifocal leukoencephalopathy; ELISA = enzyme-linked immunosorbent assay; PLEX = plasma exchange; IA = immunoadsorption.

ELISA (false positives) yet still identify samples with low levels of JCV-specific antibodies. Use of this second step to reduce false positives resulted in the lower overall seropositivity rate of 54% that we reported. Thus, the 2-step assay allowed us to maintain high sensitivity while also minimizing false positives.

The potential clinical utility of our assay for stratifying patients at risk for developing PML continues to be supported by the fact that 100% (20/20) of pre-PML samples and 100% (31/31) of samples collected at or after PML diagnosis tested positive for anti-JCV antibodies (3 additional pre-PML samples have been assayed since our paper went to press, and a manuscript on the post-PML samples is in preparation), compared with a seropositivity rate of 54% in all MS patients ($p < 0.0001$).[2] Large worldwide studies that will provide additional data on the clinical utility of our 2-step assay are ongoing.

Potential Conflicts of Interest

S.E.G., L.G., M.S.: are employees of and own stock/stock options in Biogen Idec, Inc.

Departments of [1]Neurology, [2]Molecular Discovery, and [3]Clinical Science and Technology, Biogen Idec, Inc., Cambridge, MA

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Reply

Eugene O. Major, PhD, Caroline F. Ryschkewitsch, MT, Peter N. Jensen, BS, and Maria Chiara Monaco, PhD

In commenting on JCV persistence in progressive multifocal leukoencephalopathy (PML) patients treated with natalizumab,[1] Goelz et al suggest that variation in JCV antibody enzyme-linked immunosorbent assay design leads to differing assessments of individuals' serological status, an indicator of viral exposure. With this we agree and below offer a solution. First, however, based on our CLIA (clinical laboratory improvement amendment) assay we reported 1 plasma and serum sample seronegative. These samples, from Patient 16, were taken concurrent to PML diagnosis after several cycles of plasma exchange, but exhibited antiviral titers below our interpretation of seropositive. (According to documents sent to our laboratory from the clinical site, this patient was human immunodeficiency seropositive, although this seems in question based on further information from Biogen-Idec.) We are currently unfamiliar with any systematicly derived data showing rapid reduction of JCV antibodies in patients undergoing plasma exchange (PLEX) for natalizumab removal. However, if PLEX is also responsible for JCV antibody reduction, patients who showed rising antiviral titers after PLEX, such as Patient 8, may continuously produce measureable JCV antibodies in response to viremia. Conversely, Patient 16, also viremic (85C/ml) and highly viruric (27,212C/ml), did not have a measured antibody response to JCV in our assay despite persistent antigen stimulation. Patient 16 may be an example of a seronegative PML case, not the result of JCV antibody depletion by PLEX.

Regardless of assay design, antibody cross-reactivity,[2] or viral antigen selection,[3] the assessment of serostatus could be more accurately derived from a consensus of laboratories experienced in making such assessments through independent judgment. In our paper's discussion, we urged a cross-reference of identical samples in a blinded test format by independent laboratories with data collection results sent to a third party. A similar protocol was important in evaluating the JCV DNA quantitative polymerase chain reaction assay for clinical samples, where Quality Control for Medical Diagnostics, London, United Kingdom was the objective coordinator. Such an exercise may show that the assay described in Gorelik et al[4] provides accurate data useful as 1 parameter for assessment of PML risk. However, in multiple sclerosis populations, contemplating treatment with natalizumab, or other underlying diseases, contemplating treatment with immunocompromising therapies, 1 parameter will likely not provide certainty regarding PML risk. We have publicly stated that the presence and/or rise in antibody titers could be informative when considered with evidence for viremia, T-cell–mediated immune responses, and molecular factors, all of which contribute to patients' PML risk. Now recognized more frequently, PML reaches the attention of neurologists not as a rare disease, but as a substantial consideration in the use of biological therapies that affect the immune system. In administering such therapies, measurement of several parameters would be needed to seriously mitigate PML risk.

Potential Conflicts of Interest

Nothing to report.

Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

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