Increased glutamate in CSF and plasma of patients with HIV dementia

To the Editor: The potential for glutamate levels in CSF as a useful indicator of excitotoxicity in the progression of HIV-1–associated dementia complex was recently reexamined by Ferrarese et al.1 With a larger number of patients than the original work,2 they again reported a positive correlation. Although we strongly advocate that dysregulation of glutamate dynamics in brain parenchyma may be an important component of retrovirus-induced neurodegeneration,3 our assessment of CSF found no correlation between glutamate concentration and either HIV infection or dementia.4 Ferrarese et al.1 suggested that our data may be biased by the putative degradative activity of glutaminases in CSF while frozen at −70°C.1,5 On the contrary, we directly addressed these concerns and found them to be without foundation.6 No correlation between the CSF storage times and the glutamate concentration for all individuals was evident (figure 1). In addition, no differences in glutamate levels were observed in serial measurements of individual CSF samples frozen for a period of 1 day to more than 2 years. Most importantly, we reported that the method suggested by Ferrarese et al.1 to inactive enzymes in CSF samples, exposure to 4 mmol/L perchloric acid,7 results in a nearly instantaneous hydrolysis of glutamate.4 Despite this information, CSF samples in the study by Ferrarese et al.1 were again treated in this manner (although the formula for perchloric acid was incorrectly given as monobasic phosphate).1 Consistent with an unintended hydrolysis of glutamate by this process, CSF glutamate values from subjects in the Ferrarese cohorts were 8.5- to 2-fold lower (in regards to control and HIV) than those observed in our study (3.3 μM).4 The artifacts introduced by perchloric acid exposure may not affect all samples uniformly. The apparent differences in glutamate observed by Ferrarese et al.1 may be derived from hydrolysis of additional factors, for instance, present only in the CSF of patients with HIV-1–associated dementia complex. Clearly, it would be incorrect to conclude that glutamate in the CSF from these individuals was related to levels in the brain parenchyma.

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Reply from the Authors: We appreciate the interest of Espey et al. in our works on CSF glutamate levels in patients with HIV1,2 and in our original method of glutamate determination in biological fluids.5 They agree that the discrepancies observed between our results1,4 could be explained by methodologic differences, as we immediately inactivated CSF samples with perchloric acid followed by neutralization with K2CO3, whereas they stored untreated samples. However, they propose that our method could lead to hydrolysis of CSF glutamate, which may not affect all samples uniformly. This is unlikely for several reasons. Acid-

Figure 1. Effect of perchloric acid on glutamate. Glutamate (5 μM) was exposed to perchlorate as indicated, immediately (within approximately 1 second) followed by neutralization with 100 mM potassium carbonate (final pH 10). Shown are representative chromatograph overlays of fluorescence from o-phthaldialdehyde derivatized samples following elution from a C18 reverse phase column with a 0.1 M sodium phosphate buffer containing 30% methanol (pH 5.22). The Ferrarese method (4 mM perchloric acid treatment) causes a >50% decrease in glutamate concentration due to an instantaneous hydrolysis reaction.

Figure 2. (A) Elution profile of a 100 pmol untreated glutamate solution. (B) Elution profile of the same solution after exposure to perchloric acid 0.4 M, followed by immediate inactivation with K2CO3 0.7 M. Samples were eluted through a reverse phase HPLC column by a multistep gradient of two solvents (solvent A, 0.1 M sodium acetate buffer, pH 7.2; solvent B, methanol and tetrahydrofuran 97:3 vol:vol, pH 8). Fluorimetric detection was performed with excitation and emission wavelengths of 254 and 418 nm. Glutamate peak could be clearly identified at retention time of 12 minutes, at a pH of 7.3.
treated CSF displays large increases in glutamate levels, likely linked to hydrolysis of glutamine, which is normally 100-fold higher than glutamate. For this concern, we immediately neutralized samples with equimolar K₂CO₃ (final pH 7). CSF, plasma, and glutamate solutions (figure 2) treated in this way present stable amino acid levels.

Espey et al. found no correlation between time of storage at −70°C and glutamate levels in the CSF. These data are not surprising because no enzymatic activity can be present at that temperature. Instead, the crucial factor could be the time during which CSF samples are kept at room temperature before freezing and from sample thawing to high-performance liquid chromatography (HPLC) run. Because studies performed by our group showed that it is sufficient to cause a decrease in glutamate concentration of nearly 50%, lack of inactivation of enzymatic activity in the CSF might be a source of bias in the determination of glutamate levels.

For this reason, sample acidification with or without neutralization is now employed by several groups.

Espey et al. found that when a glutamate solution is treated with perchloric acid and K₂CO₃ (their final pH is 10), the concentration of the amino acid decreases as a result of brief exposure to low pH. However, other authors suggested that alkaline condition may alter the stability of amino acid solutions.

Moreover, in our HPLC conditions, glutamate is eluted after 12 minutes at pH 7.3, while in Espey et al.’s conditions, it is eluted after 30 minutes at pH 5.2. This pH is even lower than the one that is claimed to reduce glutamate by 50% within 1 second. The HPLC run itself might then explain the glutamate decrease observed by these authors. Because they used untreated samples in their original work in patients with HIV, both the time before freezing and the HPLC run in acid conditions may bias the results.

Head circumference and incident Alzheimer’s disease: Modification by apolipoprotein E

To the Editor: Borenstein Graves et al. point to an intriguing association between small head circumference and a substantially elevated risk of AD in carriers of the ApoE-E4 genotype. They conclude that smaller head circumference leads to earlier age at onset of AD, and suggest that this distinctive relationship might reflect a smaller “brain reserve” in such individuals. Consequently, aging-associated neuronal repair mechanisms are more likely to be inadequate in individuals having a smaller head size, resulting in the observed faster AD disease progression.

We propose an alternative explanation for this observation—namely, that smaller head size might be associated with lower CNS availability of insulin or insulin-like growth factor-1 (IGF-1). Small head size at birth was recently found to be strongly associated with lower serum levels of insulin and IGF-1. Individuals with a smaller head size at birth are likely to have smaller head circumference as adults, so the connection between smaller head size and lower brain insulin/IGF-1 levels seemingly persists through adulthood. Moreover, patients with AD were found to have lower CSF insulin compared with healthy adults, an association that was more pronounced in advanced AD. In addition, recent studies have demonstrated that IGF-1 effectively protected neurons against β-amyloid–induced neuronal cell death, the major cell-death mechanism believed to underlie AD neuropathology. Together, these observations might point to a unique connection between reduced CNS insulin or IGF-1 availability and reduced protection against aging-associated neurodegenerative processes culminating in AD, a connection that could be reflected by the newly observed relationship between smaller head size and increased AD risk.

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References
Lesion selection:

Table: 5.

4. Defined as derived from table 1. expressed as a function of time after enhancement. Data weighted images and did not evolve into T1 black holes is Figure 1. Lesion evolution in placebo control and glatiramer acetate that correspond to a T2 lesion and appear hypointense. Perhaps they meant to say "scanning sequences" (e.g., T1, T1-Gd, T2), but missing even one of these sequences would eliminate the entire scan if they use all three sequences (as they describe in Materials and Methods) to determine enhancing lesions that correspond to a T2 lesion and appear hypointense on a T1-weighted scan.

3. Lesion selection: The authors identified 1722 enhancing lesions from months 1 to 6 of this study, of which 1251 (72.6%) were hypointense and were followed up. What selection criteria were used to choose these lesions? A quick review of the data in the Comi et al. publication of the same trial suggests that there were approximately 4000 enhancing lesions that occurred in this time frame (3 enhancing lesions/patient × 230 patients × 6 months).

4. Percent of patients represented in this study: It is well documented that T1 holes do not occur in all patients. Some patients with relapsing-remitting MS (RRMS) have multiple enhancing lesions and never form T1 holes. The predisposition for T1 holes may have a genetic component and patients with secondary progressive MS tend to have more T1 holes than do patients with RRMS. Therefore, it is important to know the number of patients in the placebo and glatiramer acetate groups who were followed up in this study and whether these subgroups were matched for Expanded Disability Status Scale score and disease duration. It is unlikely that the entire population is represented.

5. Table: There are a number of mathematical errors.
   a. In month 5, the total number of black holes in all patients (n = 253) exceeds that in the placebo (n = 150) + GA (n = 100) group. Similar results are shown for month 7.
   b. For months 2, 3, 5, 7, and 8, when one back-calculates the number of new lesions (column 2) from the number and percentages listed in the columns 4 and 5 (labeled placebo and GA), the total number of new lesions (column 2) does not add up to the sum of the enhancing lesions in the two groups (e.g., in month 5, there were 1084 enhancing lesions total [column 2], but I calculate 614 enhancing lesions in the placebo group + 454 enhancing lesions in the glatiramer acetate group, for a total of 1068 lesions; we’re missing 16 lesions).
   c. The authors state that they followed up 1251 lesions that were hypointense at the time of enhancement, but in month 3 there are 1543 enhancing lesions. How is this explained?

6. T1 hole volume vs T1 hole number: In the original publication of this trial by Comi et al. there was no significant difference in T1 lesion volume between placebo and glatiramer acetate groups at the end of the 9-month study. In the current article, there are fewer T1 holes in the glatiramer acetate–treated patients. How do the authors explain these data? Are there fewer T1 holes in the glatiramer acetate group, which have a larger volume?

7. Re-enhancing T1 holes: The authors state that the proportion of re-enhancing T1 holes was significantly higher in the placebo group (8.5%) than in the glatiramer acetate–treated group (5.0%) (p < 0.002). To what extent do re-enhancing holes contribute to the data, and why are re-enhancing holes even considered? Re-enhancing holes are a priori T1 holes, not hypointense lesions that have any capacity to recover.

8. Mechanism of action of glatiramer acetate: The authors state that glatiramer acetate reduces tissue damage and promotes the recovery of T1 hypointense lesions. However, if one plots out the data presented in table 1 in the original article, the recovery curves between the placebo group and the glatiramer acetate–treated group are identical until months 7 through 8, when the placebo group unexpectedly deviates downward (figure 1). If glatiramer acetate promotes lesion recovery, one would expect to see two entirely different curves from the time of enhancement. Could this deviation in the placebo group be explained by re-enhancing T1 holes? And could the effect of glatiramer acetate be to prevent this re-enhancement at months 7 through 8, when glatiramer acetate reduces the total number of enhancing lesions?

Nancy D. Richert, MD, PhD, Bethesda, MD

Reply from the Authors: We appreciate the opportunity provided by Dr. Richert’s letter to clarify a few issues that may have arisen out of a casual reading of our article and the constraints applied on its length by the review process. It also allows us to correct a few numeric errors in the manuscript that may have served to detract from our important observations on the evolution of individual MS lesions.

1. Terminology: This is in part a semantic issue. Some have used T1 hypointense lesions and black holes interchangeably—we agree with Dr. Richert’s implication that black holes is a designation that should be reserved for those hypointense T1 lesions that are long-standing and irreversible. However, we also recognized that before our study there was limited serial data on which to base a consensus definition of when to apply this term. For the purposes of our study, we defined black holes “as those lesions with signal intensity between that of the gray matter and the CSF on T1-weighted scans,” a definition that clearly includes both potentially reversible and permanent T1 hypointense lesions. We also noted in the introduction that many newly developed lesions are T1 hypointense at the time of their initial formation but only a proportion of them persist over time, and that those that are most likely to persist are the ones associated with the greatest degree of tissue destruction. Clearly, the proportion of the newly formed T1 hypointense lesions that persisted over time in our study is well delineated in the table, and can be better appreciated in figure 2. At what point in time these lesions should be considered as irreversible black holes is conjectural, but their late evolution in the placebo–treated group in the absence of re-enhancement is a novel finding of both considerable interest and concern.

2. Missing scans: Dr. Richert is correct; we wrongly reported the total number of scans. The third sentence of the results section should read: “The anticipated number of scans for the current analysis was 2390: only 122 of these (5.1%) were not available.” Although this oversights does not change the article’s message, we apologize to the readership for this mistake.

3. Lesion selection: Which lesions were selected for analysis in the current study is clearly presented in Materials and Methods.
These were lesions that arose from a previously normal white matter region and were both new on T2-weighted scans and enhancing on the corresponding postcontrast T1-weighted images. They could only be selected from sessions in which at least 3 months of follow-up imaging was available, and the lesions could not undergo re-enhancement. This lesion population is obviously a defined subgroup of the total enhancing lesions found on study as reported by Comi et al.²

4. Percent of patients represented in this study: All patients of the original trial cohort² contributed to the current analysis. Dr. Richert raises the interesting issue that persistent T1 hypointense lesions or late-evolving black holes may occur more commonly in a subgroup of patients genetically predisposed to more destructive lesions—a suggestion worthy of additional research. Because we were aware that there might be a patient predisposition to form black holes, as described in our article,¹ we performed the analysis twice, with and without correction for intrapatient lesion correlation. The two analyses yielded similar results. Future pharmacogenomic studies might better approach this issue.

5. Table: The original table contained two minor mistakes: for glatiramer acetate–treated patients, the number of black holes should have been 103 instead of 100 for month 5, and 46 instead of 44 for month 7. We cannot explain the origin of these two mistakes (the percentages in brackets in the published table were related to the correct numbers). However, this discrepancy does not affect the analysis. With these corrections, it is indeed possible to back-calculate the numbers of lesions reported for all patients in column 2 of the original table (all percentages were rounded). As regards Dr. Richert’s point 5c, we did not state that we “followed 1251 lesions, which were hypointense at the time of enhancement,” but that 1251 of the 1722 new lesions were classified as T1 hypointense at the time of their appearance. Column 2 of the original table reports the number of newly developed, nonenhancing lesions available for the analysis 1 to 8 months after their appearance. These figures must differ from that at baseline, as a given lesion could not enter the analysis if its enhancement persisted, if it re-enhanced, or if a scan time point was missing. This explains why the new lesion numbers in column 2 are higher for months 2 through 4 than for the other months (on month 1 after their appearance, many lesions were still enhancing, while lesions having only a 3-month follow-up did not have further monthly scans).

6. T1 black holes volume vs T1 black holes number: This study¹ and that by Comi et al.² are based on two completely different analyses. In this study, we evaluated the proportion of individual new lesions evolving into black holes; in that by Comi et al.,³ we measured the overall volume of T1 hypointense lesions. In the entire cohort reported by Comi et al.,³ there was a 65% relative reduction in the accumulation of mean T1 hypointense lesion volume from baseline to month 9 for the glatiramer acetate–treated compared to the placebo-treated patients that did not reach significance (p = 0.14).

7. Re-enhancing lesions: We did not report the number of re-enhancing T1 holes (as stated by Dr. Richert), but the number of identified new lesions that re-enhanced during the follow-up. By definition of what constituted a new lesion in the current study, re-enhancing lesions did not at all contribute to the main analysis of the study, i.e., proportion of new lesions evolving into black holes. This was also clearly indicated in the footnote to the original table.

8. Mechanisms of action of GA: Based on the significant reduction of the proportion of new lesions evolving into black holes, we stated that glatiramer acetate “exerts a beneficial effect on the events leading to irreversible tissue disruption once lesions are formed.” As indicated above, any difference in black holes accrual between glatiramer acetate– and placebo-treated patients cannot be attributed to re-enhancement. A plot of the proportion of newly enhanced lesions that remained as T1 hypointense lesions over time is shown in figure 2. The two curves may start to diverge after month 5; the differences become significant at months 7 and 8. Although not proven by our data, we believe that this behavior is compatible with the concept that glatiramer acetate may promote lesion recovery or retard late tissue destruction. Indeed, it would be very surprising to see an immediate effect of any drug on lesion recovery, and it would have been even more surprising for a drug like glatiramer acetate, which takes a few months to exert its effects, at least as monitored by MRI enhancement. Dr. Richert incorrectly states that at month 7 through 8, “the placebo group unexpectedly deviates.” Actually, the placebo group curve starts to deviate from month 6, while the glatiramer acetate–treated group curve steadily goes downwards (figure 2).
Clinical features of withdrawal headache following overuse of triptans and other headache drugs

To the Editor: I commend Katsarava et al.1 for undertaking the difficult task of a prospective study that involved hospitalizing a large number of patients, and for producing an incredible long-term success rate of 97%. However, I wish the authors had taken the extra step of double-blinding the trial. My concern is that their report describes clinical features of a condition that may not exist. Although withdrawal from caffeine has been shown to cause headaches in a double-blind experiment,2 the existence of withdrawal or rebound headaches due to daily use of a simple analgesic, ergotamine, or a triptan has never been demonstrated scientifically. It may be that a number of anecdotal reports have created a myth of headache from medication overuse that is not supported by facts. In the study under discussion, there are plausible alternative explanations of the observed phenomena. Hospitalizing a patient under close observation for 14 days is a major intervention that should cause improvement in a significant number of patients even without other treatment. Caffeine is another variable that must be considered. The prolonged duration of withdrawal headaches in the group of patients using combination analgesics (most of which contain caffeine) as compared with the triptan group is consistent with my clinical impression that the daily use of caffeine-containing drugs almost always causes rebound headaches, whereas daily use of triptans almost never does.

Alexander Maukpol, MD, FAAN, New York, NY

Reply from the Authors: We appreciate the interest of Dr. Maukpol in our study. Firstly, we would like to emphasize that the entity of medication overuse headache (MOH) cannot seriously be called into question. After dozens of clinical descriptions (beginning in the early 1950s,3 for review see a meta-analysis including 29 studies with 2612 patients),4 the International Headache Society (IHS) integrated MOH (initially as drug-induced headache) into its classification of 1988 as an entity of its own.5,6 However, we agree that withdrawal headache is studied less, mainly because most centers use replacement therapy during the withdrawal period. In our population, we could clearly observe an increase of headache intensity on withdrawal under the same conditions, the hospitalization itself is a blinding of the study was not possible. In contrast, Silverman et al.7 aimed to confirm the existence of withdrawal headache in an entity of its own. However, we agree that withdrawal headache is studied less, mainly because most centers use replacement therapy during the withdrawal period. In our population, we could clearly observe an increase of headache intensity on withdrawal under the same conditions, the hospitalization itself is a blinding of the study was not possible. In contrast, Silverman et al.7 aimed to confirm the existence of withdrawal headache in a placebo-controlled study. This, however, was not the issue of our study. Our goal, rather, was to characterize clinical differences of withdrawal syndromes due to different classes of drugs.

The success rate of 97% does not reflect the long-term success rate but rather confirms the diagnosis of MOH, as defined by the IHS (reduction of headache days per month of at least 50%). The long-term success is clearly lower and depends mainly on the time of the follow-up and several other aspects. The few available studies evaluating long-term success rates suggest a relapse rate of 30% to 40% in the first year after withdrawal. For our group of patients, the 1-year follow-up data are now available (unpublished data) and show a relapse rate of 35%.

We agree that hospitalization of patients under close observation may itself influence (improve) the headache. Because all patients, regardless the type of overused medication, underwent withdrawal under the same conditions, the hospitalization itself is unlikely to influence the results. Finally, as an important result of our study, we found that patients overusing analgesics (mostly containing caffeine or co-

References


May (1 of 2) 2002 NEUROLOGY 58 1443
deine) had a longer and more severe withdrawal than patients overusing ergots or triptans. Whether the additional withdrawal from caffeine and codeine worsened the withdrawal symptoms is an interesting aspect that cannot be excluded but warrants further study.

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References

Rapid infusion of intravenous immunoglobulin in patients with neuromuscular diseases

To the Editor: Grillo et al. reviewed the use of rapid infusion of IV immune globulin in patients with neuromuscular disorders. Their abstract and discussion claim the safety and convenience of this practice in their population of patients, and the final sentence of their abstract states: “Rapid infusion IVIg can be given safely and conveniently in many patients with neuromuscular disorders.” Although this is accurate for the majority of their patients, the authors report 89 adverse events in 341 rapid infusions in 50 patients, 3.5% of which were considered “major.” This amounted to a major event in 11 out of 50 patients (22%).

These major events and their frequency are of concern to us, as these events included chest pain, myocardial infarction, congestive cardiac failure, severe headache requiring hospitalization, pulmonary embolism, and “transfusion related acute lung injury.” These serious occurrences are certainly related directly to the rapid infusion protocol (reaching as high as 800 mL/hour) in what is essentially an at-risk population. Some of these adverse events are noted in the product information insert for the product used, and our own recent analysis of them, reported via pharmacovigilance, has identified rapid infusion of IV immune globulin as a possible risk factor.

It is strongly recommended that clinicians and other health care workers such as pharmacists and nurses who may be associated with the therapeutic administration of IV immune globulin read and understand the product insert and follow the noted recommendations related to the rate of infusion of this therapeutic agent.

Edward D. Gomperts, MD, Fred Darr, MD, Glendale, CA

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Reference

Correction

Giant axonal neuropathy (GAN): Case report and two novel mutations in the gigaxonin gene

In the article, “Giant axonal neuropathy (GAN): Case report and two novel mutations in the gigaxonin gene” by Kühlenbaumer et al. (Neurology 2002;58:1273–1276), there was an error in the Results section, under the subsection “Molecular genetic analysis” (page 1275, line 18). “One of the patient’s brothers (II-3) inherited the exon 3 nonsense mutation from the mother” should read “… from the father.” The authors apologize for this error.
Giant axonal neuropathy (GAN): Case report and two novel mutations in the gigaxonin gene

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