

Intranasal VIP safely restores volume to multiple grey matter nuclei in patients with CIRS

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Abstract:

Evidence has accumulated since 2008 that intranasal, topical application of vasoactive intestinal polypeptide (VIP) can be administered safely to patients with a multi-system, multi-symptom illness, called chronic inflammatory response syndrome (CIRS), acquired following exposure to biologically produced toxins and inflammagens. To date, over 300 physicians have prescribed intranasal VIP for their CIRS patients; these physicians have overwhelmingly (>90%) reported intranasal VIP to reduce symptoms; reduce elevated levels of MMP9, TGF beta-1 and C4a; raise low levels of VEGF; normalize clotting abnormalities, including acquired von Willebrand's; and return regulation to pituitary systemic axes involving ACTH/cortisol and ADH/osmolality. These changes did not occur without use of VIP, though antecedent steps in a sequential treatment protocol provided benefit for many patients. The mechanism of these salutary health effects provided by VIP likely involves correction of abnormalities in ribosomal gene activation, nuclear encoded mitochondrial gene activation and an upregulation of Ikaros, a zinc fingered transcription factor. Recent preliminary work using sequential measurement of CNS volumes by NeuroQuant showed a longer treatment window (> 12 weeks) and higher doses of VIP (> 6 doses/day) safely corrected multinuclear atrophy of grey matter. The current study built upon this preliminary work and identified a course of VIP treatment that restores grey matter nuclear atrophy in CIRS patients. Safety of VIP and durability of benefit were both shown with no significant adverse effects reported by any patients.

Key words: VIP, proteomics, transcriptomics, grey matter nuclear atrophy, sarcin-ricin loop, ribotoxins, Ikaros

Background: Chronic inflammatory response syndrome (CIRS) is a chronic, progressive, multi-system, multi-symptom syndrome characterized by exposure to biotoxins, HLA genetic predisposition, altered innate and adaptive immunity, peripheral hypoperfusion at multiple sites and multiple hypothalamic-pituitary-end organ dysregulations [1]. This inflammatory dysregulation can affect virtually any organ system of the body; if left untreated, can become debilitating. In many instances of CIRS, even when patients are removed from the offending environment and treated with a stepwise protocol, their condition does not improve to the pre-morbid state. The most prominent feature of these treatment resistant patients is their abnormally low level of plasma VIP [1]. The clear majority of these patients (> 90%, unpublished) show a marked improvement and reduction in symptoms after use of intranasally applied VIP as the final step in the sequential treatment protocol.

Vasoactive intestinal peptide (VIP) is a 28-amino acid neuropeptide that has been studied extensively since first isolated by Said in 1970 [2]. The patients in this study were administered synthetic VIP, identical to human VIP, compounded by Hopkinton Drug, Hopkinton, Massachusetts. This compounding of VIP undergoes rigorous control steps to verify stability and purity, to make sure safety in use by humans is maintained. VIP has been used since the early 1980's to treat asthma [3, 4] and pulmonary hypertension [5-7]. Of note, a recent clinical trial of VIP in patients with chronic inflammatory response syndrome (CIRS) used a rise of pulmonary artery systolic pressure (PASP) in exercise > 8 mm Hg as an inclusion criterion [1]. As all patients were shown to have reduction of a rise in PASP during exercise after use of VIP, these patients were identified as having acquired pulmonary hypertension. With use of VIP alone but without use of the sequential

protocol, patients did not exhibit the reduction in rise of PASP in exercise.

To understand the molecular mechanisms of VIP in these treatment resistant CIRS patients, transcriptomic sequencing (mRNA-Seq) was used to identify changes in gene expression [8]. This differential gene expression was then used to identify molecular pathways changing in response to VIP treatment, changes that paralleled patient improvement. The most prominent changes in gene regulation were found in ribosomal and mitochondrial activity. Ryan et al, went on to discuss the role of microbial toxins that cleave or modify ribosomal RNA at the conserved sarcin-ricin loop in the functional ribosome, rendering the ribosome ineffectual. In addition, the authors posited that since the mitochondria have their own ribosomes, mitoribosomes, also containing the evolutionarily conserved sarcin-ricin loop, these mitoribosomes may also be vulnerable to microbial toxins.

It is not known how VIP is acting to influence ribosomal or mitochondrial gene regulation, but several transcription factors were also shown to be differentially expressed after administration of VIP. One of these transcription factor families, Ikaros, was shown to be the most highly up-regulated assemblage of genes as determined by Gene Set Enrichment Analysis using Fishers Exact Test. All five isoforms of Ikaros were up-regulated after VIP treatment, which is crucial to stemming proliferative stages of T and B cells [9]. It appears the VIP/Ikaros relationship may be one of positive feedback. This would explain why the absence of VIP signaling in these patients corresponded with low levels of Ikaros gene expression since Ikaros has been shown to upregulate VIP receptor expression through 12 putative binding sites in the promotor regions of each of the two VIP receptor genes [10].

In parallel, CNS volumetric studies in CIRS patients using the brain MRI volumetric quantization software NeuroQuant (NQ) identified a pattern of volume abnormalities seen in CIRS-WDB patients but not others [11]. The concordance of these unique findings with CIRS-WDB illness was near 100%. A later volumetric study following use of sequential treatment protocols showed resolution of interstitial edema in forebrain parenchyma, cortical grey and pallidum, but not atrophy of caudate [12].

There is extensive literature demonstrating penetration of intact VIP across the blood/brain barrier [13, 14] as well as evidence of neuroprotection that prompted consideration of VIP in Parkinson's [13] and Alzheimer's [15, 16]; [17]. In this open label study, we collected serial CNS volumetric scans before and after initiation of VIP, seeking proof of concept that use of VIP could affect grey matter volume. All patients in this study met a strict case definition produced by the US GAO [18]; all patients were treated with the sequential treatment protocol for CIRS prior to receiving VIP; no patients reported adverse effects. Current studies in progress will continue to expand delineation of safety and diverse health benefits of use of this anti-inflammatory, regulatory neuropeptide.

Methods: VIP was compounded by Hopkinton Drug, Hopkinton, Massachusetts, to 500 mcg/mL, providing 50 mcg/0.1 ml as single dose. The peptide was dissolved in sterile saline for irrigation, to which 1% glycerin USP was added to prevent aggregation and to help preserve the structure of the protein. All glassware and equipment used were disinfected with 70% isopropyl alcohol prior to use and rinsed with sterile saline solution. The nasal sprayer used is

manufactured by Aptar Pharma and is compliant with all requirements of USP <601>. The product, despite passing a 90-day stability study, is dispensed with a 60 day beyond usable date (BUD) under refrigeration to guard against possible failures in patient compliance with handling requirements. Patients are instructed to keep the product under refrigeration at such a temperature that repeated freezing and thawing won't occur. The pH of the finished solution was between 6.1 and 6.2. To determine consistency of the compounded preparation, the pH was tested over a 10-week period while kept under refrigerated conditions as recommended to patients by Hopkinton Drug. Given the predicted isoelectric point of the peptide (9.82 – calculated with ExPASy pI calc.) a pH of ~6 is advantageous for both stability of the peptide and suitability of the solution as a nasal spray.

The VIP peptide is prepared by conventional solid-phase chemistry, with residual solvent content of DMF, TFA, methylene chloride, and acetonitrile well below acceptable range per United States Pharmacopeia (USP) <497>. A separate lot of VIP was pulled and tested for these impurities concurrent with patient dispensation. In addition, the lot was tested by circular dichroism for peptide confirmation.

Patients:

As in all studies of VIP performed in CIRS patients, (1) all patients met the established case definition [18] for chronic inflammatory illness. (2) Differential diagnosis did not reveal confounders. (3) All patients followed a standard sequential treatment protocol before VIP therapy was begun. (4.a) All patients had confirmation of exposure to buildings with abnormal mold specific qPCR (MSQPCR) scores (ERMI or HERTSMI-2); (4.b) absence of biofilm forming multiply-antibiotic resistant coagulase negative

staphylococci (MARCoNS) resident in deep aerobic nasopharyngeal space; and (4.c) normal measures of visual contrast sensitivity (VCS). (5) All patients had normal levels of lipase before the VIP treatment trial began.

35 CIRS patients, ranging in age from 19-64, 14 males and 21 females, with persistent proteomic and transcriptomic abnormalities, despite use of a standardized treatment protocol, were enrolled in the study and prescribed VIP. Four patients were unable to obtain VIP in the states they resided because of state specific pharmaceutical distribution regulations. However, these patients remained in the study as negative controls and received the same MRI scans and NeuroQuant measurements as patients on VIP. Deidentified NQ General Morphometry Reports and MRI results were supplied to a single clinic performing the group NQ analysis, looking solely for changes in volume, especially grey matter nuclear volumes. Control data on 10 different CNS structures were compared to values of patients at baseline. Patient values were considered abnormal for any structure if they exceeded a difference of 1.45 standard deviations (std) from controls. The selection of 1.45 std's represents volumes that fall outside of 85% of normal values, or stated another way, only a 0.15 probability the value is from a normal population. This value is even more significant in the context of our search for atrophy in that a one tailed approach using standard deviation means that there is only a 0.075 probability the value is from a normal population, or 92.5% chance the value is not from a normal population. We feel this is medically significant and requires monitoring.

Values of cases were then analyzed for differences after VIP, with change of at least 1 standard deviation after treatment considered significant, either for improvement or worsening. We feel a 1 std shift is outside

of typical measurement fluctuation and indicates a change in biology. Data were analyzed retrospectively under a waiver approved by Copernicus Group IRB, Research Triangle Park, NC.

Patients enrolled in the trial received a single test dose of VIP, one spray consisting of 50 mcg/100 mcl, was given in one nostril while being monitored in the medical office after baseline lab testing was drawn. Patients were then allowed to self-administer their VIP as they monitored possible side effects. Recommended titration of VIP dosing began with one spray given four times a day for one month, increasing to two sprays four times a day each month if symptoms were not resolved by the end of the first month of VIP treatment. Patients were allowed to stay on VIP as long as they felt improvement or maintained stability. There were no dropouts for adverse events during the clinical trial. For analysis, patients were stratified by age, dose and duration of treatment.

NeuroQuant® (NQ):

Specialized volumetric studies were performed by radiology suites using NeuroQuant (NQ) software (Cortechs Labs, <http://www.cortechslabs.com/neuroquant/>).

For each patient, a brain MRI was performed before use of VIP. Since 2006, when the FDA cleared the Cortechs software protocols for use commercially, there has been no evidence of variations in reporting from site to site; or machine to machine; or due to differences in coil size from 1.5 Tesla to 3.0 Tesla, that could independently alter the volumes recorded by the software. Measurements were recorded as a percentage of intracranial volume, both left and right hemispheres, from ten areas including: forebrain parenchyma; cortical grey; lateral ventricle; hippocampus; amygdala; caudate; putamen; pallidum; thalamus and cerebellum. Volumes of inferior

lateral ventricle were not included in analysis as little change in volume comparing cases to controls was observed. Volumes for these 20 areas were compared to a clinic-specific control group, mean age 49.6 for women and 41.9 for men, as previously reported [11].

Absence of anti-VIP antibodies:

Because VIP is a peptide, and antigenicity is always possible, concern was present regarding the potential adverse effect of VIP inducing anti-VIP antibodies. We relied on an FDA risk assessment tool, Guidance for Industry; Immunogenicity Assessment of Therapeutic Protein Products, to seek clinical evidence of safety. We sought to answer a published series of questions regarding clinical problems developing after use of peptide or protein products.

1. Was there anaphylaxis?
2. Was there consistent evidence of cytokine release syndrome?
3. Were there infusion or topical use reactions?
4. Were there non-acute reactions?
5. Did we see evidence of cross-reactivity to endogenous proteins?

Since the answer to each of these questions was no, we did not pursue additional studies for evidence of anti-VIP antibodies.

Results:

There were no adverse events that caused withdrawal of any of the 35 patients who used VIP. There were no significant gender-related differences in mean volumes shown by NeuroQuant. Sources of variability in dosing was multifactorial, with access, cost and convenience the most common reasons. Laboratory studies were not uniformly supplied to the clinic so no attempt could be made to show concordance of resolution of grey matter nuclear atrophy with improvement in labs.

VIP peptide stability analysis

Testing for pH stability showed the VIP solution was pH stable for at least 90 days (Table 1).

Additionally, analysis of VIP synthesis showed all contaminants to be well below permitted daily exposures as set forth by USP (Table 2). The peptide was confirmed to be 98.8% free of impurities, including peptide fragments and incorrectly coded peptides, as shown by analytical HPLC. The largest single impurity is 0.4% of the peptide. Stability of VIP in saline solution, pH ~6.15, is over 90 days as shown by a stability study performed by Compounder's International Analytical Laboratory of Castle Rock, CO using HPLC. The same lab also confirmed that compounded VIP nasal spray passes endotoxin, fungi, and sterility testing. The reason for compounding VIP in nasal preparation was to avoid rapid lysis by endopeptidases in whole blood [19, 20] and by hepatic metabolism [21]. Product integrity was shown repeatedly by Compounder's International Analytical Laboratory (2012, 2013, and 2014) and private HPLC testing using an Agilent 1260 chromatograph (2016) without evidence of increase in incorrectly coded peptides or VIP degradation in its atomizer. Circular dichroism (CD) of a lot (10282016: 50@14) of VIP using a Jasco J-1500 spectropolarimeter showed increasing helical content with increasing solvent alcohol content, reaching 85% α -helix in 50% methanol (Alliance Protein Laboratories, 11/1/2016). Because far UV CD determines the secondary structure due to asymmetric environments of the peptide, the CD result is consistent with what was expected.

Data on long-term use of the compounded preparations were kept by physician surveillance and inquiry regarding possible adverse effects as each prescription was refilled. Beginning with one physician

ordering the product in 2008, use has extended now to 314 physicians with over 7000 prescriptions filled. No data has emerged to suggest toxicity potential of the product.

Reduced Nuclear Volumes

Five patients showed enlarged lateral ventricles ($> 0.96\%$ mean ICV), a condition that suggested presence of pathology not responsive to VIP by prior anecdotal observations. Given the differences in clinical presentation (older, more atrophic nuclei), and absence of change of grey matter nuclei in patients with enlarged lateral ventricles with any therapy in several years of data recording, we elected to create a separate category for the five patients with enlarged lateral ventricles, leaving 26 for whom comparison was made to the five enlarged lateral ventricle patients and four no-VIP use controls.

There were 13 patients with low dose, less than 7 doses per day, for up to three months; and 13 patients on higher dose regimens (≥ 7 doses per day) for up to six months use of VIP. The small numbers of patients in the study prevented further stratification of dose over time.

There was no difference in mean volumes of the 11 brain structures before use of VIP by gender. We observed correction of multinuclear volume reduction of grey matter nuclei in a heretofore unreported percentage of patients.

Because we have already presented data showing improvement in forebrain parenchyma and cortical grey areas [12], using steps of the sequential protocol that stopped short of using VIP, we confined our analysis to volumes of left and right hemispheres separately for grey matter nuclei

including hippocampus, amygdala, caudate, putamen, pallidum, thalamus; and cerebellum, for a total of 14 data points for each patient. For patients who did not take VIP (four patients, each with 14 data points), 48 of the 56 total structures measured (85.7%) either did not improve or were worsened in size $>$ one standard deviation (Table 3, overall change with treatment) while 8 (14.3%) improved in volume. These data are consistent with the observation that grey matter nuclear volume loss in CIRS patients rarely self-heals. For those with enlarged lateral ventricles, 66 of 70 measurements (94.3%) showed worse or no improvement in volumes; 4 of 70 (5.7%) improved, though none reversed frank atrophy. These data are in line with anecdotal observations that enlarged lateral ventricles seen in patients ≤ 65 years of age rarely show improvement in grey matter nuclear volume with any therapy.

For the 13 patients who took VIP of variable duration, in those using it for 0-12 weeks, 28 of 182 (15.4%) structures showed improvement of volumes > 1 standard deviation, with 120 of 182 (66.6 %) unchanged and 34 of 182 (18.7%) worsening. (Table 3). For the 13 patients on higher dose and longer duration of VIP, >12 doses per day, with some up to of 24 weeks, 48 of 182 (26.4%) were improved, 98 of 182 (53.8%) were unchanged, and 36 of 182 (19.8%) worsened, with reduction of overt atrophy to 0.9%, a level equal to that of controls. While 35.7% of structures in patients who didn't take VIP worsened, suggesting the active atrophying process continued aggressively, use of VIP in each of the three (LV, low dose, higher dose) treatment groups showed slowing of volume loss (14.3-19.8%).

A comparison of total abnormalities, with standard deviation ≥ 1.45 , in cases compared to controls (Table 4, total abnormalities),

showed benefit of VIP at low dose being 22.2 % and higher dose 47.3%.

Focusing on grey matter nuclei

Reporting individual grey matter structures showed new findings for each of our four groups (Table 5, % improvement after VIP). Restricting data assessment to seven grey matter structures reduced some possible confounding variables by eliminating forebrain parenchyma and cortical grey, structures that are enlarged in cases; and lateral ventricle, a structure that rarely changes significantly. The No-Treatment group (No-RX, N=4) was too small to show significant increase in size of hippocampus, amygdala, caudate, putamen, pallidum and in thalamus. The lateral ventricle group (LV, N=5) similarly showed no significant improvement in any grey matter structure. For the larger treatment groups (N=13 each), low dose Rx gave improvement in 7.7% of hippocampus; 38.5% of amygdala; 15.4% of caudate; 38.5% of putamen and pallidum; 46.1% of thalamus; and 20% of cerebellum. High dose Rx yielded increases of 61.5% in hippocampus, caudate and putamen; 15.4% of amygdala, 30.8% in pallidum, 76.9% in thalamus; and 53.8% of cerebellum.

Discussion:

This open label trial was designed to examine the possibility that use of VIP could reverse grey matter nuclear volume reduction in CIRS patients with inadequate response to the initial series of sequential steps of standard treatment protocols. A secondary goal was to test the use of intranasal VIP for evidence of safety and efficacy. To date, this is the third trial by our group showing benefit of VIP in CIRS patients; each also shows no evidence of adverse effects of the drug. These data build on the expanding literature on CIRS, showing parallel findings in the proteomics of chronic ciguatera [22]; genomics of ciguatera

[23]; confirmation that CIRS creates a fingerprint of CNS volumetric injury [11]; a finding corrected in part by use of earlier steps of the sequential protocol [12] showing efficacy in reduction of microscopic interstitial edema seen in CIRS patients.

Comparing the published data on the pharmaceutical evidence underlying use of VIP as a nasal spray [15] to data supplied by the compounding pharmacy for these patients, we see all necessary criteria met. The compounded liquid (1) was chemically and biologically stable, (2) was confirmed to be the correct pH of 6.2 over recommended use period; (3) was confirmed to have the correct ionic strength; (4) had an acceptable concentration; (5) contained an absence of solvent contamination; (6) contained an absence of significant amount of peptide impurities; (7) showed no clinical evidence of formation of anti-VIP antibodies; (8) demonstrated expected alpha helical structure of VIP protein in solution.

Since we did not employ a method to identify presence of anti-VIP antibodies (AVA), we looked for discussion of AVA in published literature. We found one reference to two patients reported in a letter to ATS [24] in which the authors promised publication of details on these patients apparently treated with VIP. No such subsequent publication has been identified. We cannot place weight on this letter, particularly with our 8-year experience with this peptide and its intranasal delivery system. Veljkovic reports [25, 26] that physical activity can increase levels of AVA in patients who exercise habitually. Indeed, he cites Said as showing production of AVA to be found in 29.6 of exercisers. Such production is thought to be protective from effects of excessive VIP, a condition not found in CIRS patients. The likelihood that CIRS patients could exercise to levels

discussed by Said is remote, as most have excessive daily fatigue [1] and low VO₂ max (unpublished).

The improvement in size seen simultaneously in multiple grey matter nuclei is unparalleled in current literature. Current NQ reports focus on reduction of hippocampal volume as an indicator of Alzheimer's, yet that reduction in size is ameliorated in over 60% of VIP-treated patients. We feel that even in Alzheimer's disease there is room for discussion of possible use of VIP to increase plasticity and response to regulation of DNA transcription, together with reduction of peripheral proteomic markers of innate immune activation. Of concern is the possibility, as seen in amygdala, that high dose VIP could possibly aggravate volume loss. Further studies, prospectively performed are underway to correlate transcriptomic findings and changes in NQ with structured treatment with VIP at the end of a published, peer reviewed treatment protocol [1].

Of importance in this report is the published literature showing safety and efficacy for use of intranasal VIP to provide delivery of VIP to the brain [13, 14, 20]. Moreover, the theoretical basis for consideration of VIP to reduce grey matter atrophy is also supported [27]. In fact, Cui [15] cites VIP as possibly able to treat Alzheimer's disease; Dogrukol suggests that VIP could treat Parkinson's disease, as VIP crosses the blood-brain barrier. Morell [16] suggests that VIP has an immunomodulatory and neuroprotective role in the CNS (see this reference for an overview of VIP in normal adult brain physiology; and VIP in neuroinflammatory diseases). Morell also supports safety and efficacy of VIP, citing studies in pulmonary hypertension and sarcoidosis, but that drug delivery by systemic application is reduced by endopeptidases [19, 20] and by hepatic metabolism [21]. Use of

nasal application avoids reduction of efficacy by endopeptidases.

VIP is shown herein to safely achieve reduction, and indeed, less commonly, resolution of multinuclear grey matter depletion to equal controls in a small subset of higher dose users. Prior work has shown durability of safety and efficacy of VIP [1]. With transcriptomics showing that VIP corrects abnormalities in essential cellular processes, we may hypothesize that the replenishment in grey matter nuclear volumes involves ribosomal and mitochondrial changes in CNS neurons.

In the past, attempts to correct CNS atrophy using nerve growth factors [28-31] has not been successful, raising the issue of presence of an inhibitor that blocks axonal or Purkinje fiber regrowth. The concept then extends to compounds like glial fibrillary acidic protein as such an inhibitor. Opposing such an inhibitor by agents such as Kruppel-like factor 4 [32] that have that capability is intriguing. Unpublished data from the Ryan 2016a paper shows that VIP upregulates KLF4 by a factor of 1.3.

Conclusions:

This report adds to the rapidly expanding published literature on use of VIP in a diversity of medical conditions. We have not identified any previous paper that shows correction of multiple grey matter nuclear injury as shown by reduction intracranial volume. Understanding that we need to complete a double-blinded prospective clinical trial, we are heartened to find that correction of more than 25% of atrophic nuclei seen in seven grey matter structures suggests that correction of both inflammatory responses and genomic responses by VIP could provide a new approach beyond CIRS to treatment of degenerative CNS illnesses,

including early onset dementia. For those with cognitive decline in the face of CNS atrophy, normally considered to be irreversible, we can now reasonably suggest the possibility of new avenues of treatment.

There is wealth of published literature showing anti-inflammatory and immunomodulation effects of VIP both peripherally and centrally. In its role as a primordial transcription agent, VIP has the capability of affecting countless metabolic pathways and their interactions in ways not fully established [33]. Understanding that we do not have all the answers regarding use of VIP does not prevent us from pursuing well-established clinical benefit in patients with profound disability from illnesses with a

multiplicity of names from Chronic Fatigue Syndrome to fibromyalgia; and PTSD to CIRS. We have shown safety in pharmaceuticals and safety in clinical medicine. We have used sophisticated objective measures to show unequivocal benefit. As we expand our research on and clinical use of VIP, unrecognized pathways and benefits are likely to be developed. As we build on the insights of VIP researchers like Sami Said and Mario Delgado, we will add successful therapy of countless illnesses that remain the scourge of the 21st century.

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Tables

VIP Production Date / First 8 digits of Lot#	pH measured 10/24/2016*
10/24/2016	6.2
10/20/2016	6.1
10/10/2016	6.2
9/27/2016	6.2
9/19/2016	6.2
9/6/2016	6.2
8/25/2016	6.1
8/16/2016	6.1

*Measured using Horiba LAQUAtwin pH meter. Two point calibration (pH 4, 7) performed before and between each measurement.

Table. 1 – pH stability of compounded VIP nasal spray

SOLVENT / REAGENT	Hop. Drug VIP MAXIMUM DAILY EXPOSURE (mcg/day)*	USP 467 MAXIMUM PERMITTED DAILY EXPOSURE (mcg)
Acetonitrile	0.188	4,100
Trifluoroacetic Acid	1.6	(none listed)
Dimethylformamide	0.584	8,800
Dichloromethane (Methylene chloride)	0.568	6000
SOLVENT / REAGENT	Hop. Drug VIP MAXIMUM CONC (ppm)	USP 467 MAXIMUM PERMISSIBLE CONC (ppm)
Acetonitrile	47	410
Trifluoroacetic Acid	400	(none listed)
Dimethylformamide	146	880
Dichloromethane (Methylene chloride)	142	600

*based on 60 sprays / day

Table. 2 – Solvent / reagent concentrations in compounded VIP nasal spray. (Note: The lot of bulk API used was produced before the implementation date of USP <503.1> - Trifluoroacetic Acid in Peptides. Due to the route of synthesis used, the manufacturer of the bulk API is confident that the vast majority of residual TFA would be in the form of the less hazardous acetate salt. 400ppm is the lowest detectable amount for the HPLC used)

Four treatment groups are identified: No RX= no VIP; LV= enlarged lateral ventricle
Low dose= up to 6 sprays a day for up to three months; high dose= more than 6 sprays for up to
six months

Table 3				
Group Name	N=	% Worse	% Same	% Better
No RX	4	35.7	50	14.3
LV	5	14.3	80	5.7
Low Dose	13	18.7	66	15.4
Higher Dose	13	19.8	53.8	26.4

Overall change with treatment

Table 4				
Group Name	N=	Abn	Corrected	Worse
No RX	4	7.25	2.75	0.75
LV	5	9.2	1.4	0.8
Low Dose	13	7.36	1.64	1.07
Higher Dose	13	10.18	4.82	0.18

Total abnormalities > 1.45 SD after treatment

Table 5								
Group Name	N=	Hippo	Amygdala	Caudate	Putamen	Pallidum	Thalamus	Cerebellm
No RX	4	0*	50*	0*	50*	25*	25*	50*
LV	5	0*	20*	0*	20*	0*	20*	25*
Low Dose	13	7.7	38.5	15.4	38.5	38.5	46.1	30.8
Higher Dose	13	61.5	15.4	61.5	61.5	30.8	76.9	53.8

% Improvement after VIP

*** groups are too small for calculation**