Gene Deletion of VIP Leads to Increased Mortality Associated with Progressive Right Ventricular Hypertrophy

Anthony M. Szema, MD, * and Sayyed A. Hamidi, MD

Abstract

Vasoactive Intestinal Peptide (VIP) knockout mice exhibit asthma, pulmonary hypertension, and left ventricular wall thinning. Humans with these disorders have premature death. We show here that VIP KO mice have reduced survival (100% mortality at 20 months), vs. 100% survival among WT C57BL/6 mice. Moreover, the ratios of weights of right ventricle divided by left ventricle plus septum were progressively increased in VIP KO mice with age. Core temperatures were lower in VIP KO mice when compared to WT littermates, with an associated pro-inflammatory cytokine milieu. Overall, our results indicate that VIP is important for survival in mice. Its absence leads to increased mortality, with progressive right ventricular hypertrophy as a surrogate of pulmonary hypertension, lower body weight, hypothermia, and pro-inflammatory milieu. These studies support VIP as a novel therapeutic agent in pulmonary hypertension.

Keywords — asthma, knockout mice, pulmonary hypertension, right heart failure, Vasoactive Intestinal Peptide, VIP.

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I. INTRODUCTION

In the fourth decade since the discovery of Vasoactive Intestinal Peptide (VIP) by Sami I. Said, MD, and Victor Mutt, PhD, our understanding of the function of VIP in human disease has undergone a radical transformation from that of a smooth muscle relaxant and inhibitor of smooth muscle proliferation to new anti-inflammatory and immune-protective properties including: 1) upregulation of anti-inflammatory cytokine IL-10 and 2) induction of protective regulatory T cells (T-reg).

Synergistic properties of muscle modulation and immune control may explain the concomitant phenotypes of asthma, pulmonary hypertension, and heart failure observed in mice with targeted deletion of the VIP gene (VIP knock-out mice or VIP KO). In the present study, we sought to determine the effects of VIP gene deletion in mice on survival, ventricular hypertrophy core body temperature, and regulation of pro-inflammatory cytokine genes associated with death, weight loss, and hypothermia, including tumor necrosis factor alpha (TNF-α) and IFN-γ.

TNF-α functions as an endogenous cryogen, so upregulation of TNF-γ gene expression in VIP KO mice would support the concept of hypothermia in these animals, especially since VIP is known to upregulate IL-10, leading to hypothermia attenuation. In a model of colitis, treatment with VIP reduced clinical and histopathological severity, abrogating body weight loss, diarrhea, and macroscopic and microscopic intestinal inflammation.

The therapeutic effects of VIP were associated with down-regulation of both inflammatory and Th1-driven autoimmune responses, including TNF-α, interleukin 1, and interleukin 6 in colon extracts and serum as well as interferon gamma in splenic and lamina propria CD4+ T cells. VIP reduced disease severity when given after disease onset and dramatically reduced disease recurrence following a second dose of trinitrobenzene sulfonic acid (TNBS) used to induce colitis.

VIP is also known to prevent programmed cell death (apoptosis), therefore lack of VIP would logically render an animal more susceptible to this pathway. Indeed, VIP stimulates astrocytes to secrete the thrombin-inhibiting neurotrophic serpin, protease nexin I, preventing thereby activity-dependent neuronal cell death. In addition, ovary – VIP interactions also inhibit apoptosis, suggesting that loss of the VIP gene could have deleterious consequences in multiple organ systems, supporting the concept of whole organism adverse outcomes.
II. METHODS

The study was approved by the IACUC of the Northport VA Medical Center (New York, USA).

A. Mice

15 male wild-type (WT) C57BL/6 mice and 38 VIP KO littermates (backcrossed to C57BL/6) were housed in a triple barrier isolation facility and fed mouse chow ad libitum.

Kaplan Meier plots were constructed based on age of natural death. In case of rare severe spontaneous wounds, after consultation with the Veterinary Medical Officer, mice were humanely euthanized. Mice were monitored by veterinary staff daily and by the investigators 2 times a week.

B. Statistical Analysis

For mortality data, Kaplan-Meier curves were generated and compared using the log-rank test.

All analyses were performed with STATA software (Stata Corp, LP, College Station, TX), and a $P<0.05$ was considered statistically significant.

C. Gene Microarray

In a separate series of experiments with mice not involved in the mortality or right ventricular hypertrophy study. For gene microarray analyses, RNA was isolated from lung samples of 5 male VIP KO and 5 WT mice and subjected to Affymetrix gene profiling (Expression Analysis, Durham, NC).

The aim was to search for significant differences between the 2 groups in the expression of genes relevant to inflammation and cachexia or its converse, obesity.

D. Temperature:

Temperature was measured in 11 VIP KO and 7 WT mice with an infrared thermometer.

E. Right Ventricular Hypertrophy:

In a separate series of experiments with mice not involved in the mortality or gene microarray studies, the heart was isolated and placed under a dissecting microscope for anatomic assessment of RV Hypertrophy. Attached vessels and both atria were dissected and removed. The RV wall was cut out, blotted, and weighed; then the left ventricular wall and septum (LV+septum) were treated the same way and weighed. The RV/(LV+septum) ratio was calculated in 31 male VIP KO mice and 8 male WT littermates as an index of RV hypertrophy. To evaluate the differences in vascular pathology between male and female mice, we also assessed RV mass as measured by the mean RV/(LV+septum) weight ratio in male VIP KO mice and male WT mice. Mice were euthanized with high dose pentobarbital prior to obtaining tissue.

F. Body weight:

To assess the weight, 63 WT and 49 VIP KO mice were weighed on a scale at respective ages.

III. RESULTS

Kaplan-Meier survival estimates revealed that control WT mice had 100% survival (no mortality) among 25 mice at 20 months. In contrast, the VIP KO mouse line exhibited reduced survival with no mice alive by 20 months. Beginning at 5 months, mortality was evident and by 7 months 50% lethality was observed (Figure 1).
The pulmonary vascular pathology is progressive in VIP KO mice with age-related increasing right ventricular hypertrophy and ratios of right ventricle divided by the sum of the weight of the left ventricle plus septum (RV/LV+septum). Whereas WT mice even at the oldest ages have RV/(LV+septum) ratios of 0.20 between 72-106 weeks in Figure 2, in contrast, VIP KO mice have higher and increasing ratios with age, ranging from 0.30 at 5-18 weeks, 0.36 from 30-38 weeks, and 0.37 from 51-143 weeks. So, an association exists between age-related mortality and progressive RV hypertrophy in VIP KO mice compared to WT mice, which do not exhibit RV hypertrophy at even the highest ages and do not have susceptibility to mortality younger than 20 months.

Body weight was consistently lower among VIP KO mice versus control littermates at all ages (Table 1). For instance, between 6-14 weeks the average weight was 23.8 grams in VIP KO compared to 28.8 grams in WT mice. With time, weights increased, with VIP KO 26.9 grams at 15-19 weeks and WT continuing to be higher at 34.1 grams. At age 20-29 weeks, WT average weights were 32.6 grams, while VIP KO mice were 27.5 grams. At 34-36 weeks, the weights were similar within both groups, VIP KO and WT averaging 31 grams. Recall that these VIP KO mice alive at 34-36 weeks are those who were still living, since we did not weigh deceased mice. Body temperature was found to be consistently lower among VIP KO mice compared with WT mice.

We earlier reported airway inflammation and hyper-responsiveness similar to human asthma, with high concentrations of pro-inflammatory cytokines IL-6 and IFN-γ in bronchoalveolar lavage from VIP KO mice. These are key cytokines associated with weight loss, hypothermia, cachexia, and death.

The leptin gene (Figure 3) was upregulated 2.28 fold in VIP KO mice compared to WT mice and IL-6, IL-1 alpha and beta were increased 1.42, 1.69 and 1.76 fold, respectively (Table 2).

## Table 1: Comparison of body weight between WT and VIP KO mice, stratified by age groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>WT (n)</th>
<th>KO (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-14 wk</td>
<td>28.8 (17)</td>
<td>23.9 (9)</td>
<td>0.0018</td>
</tr>
<tr>
<td>15-19 wk</td>
<td>34.1 (6)</td>
<td>28.9 (6)</td>
<td>0.001</td>
</tr>
<tr>
<td>20-29 wk</td>
<td>32.6 (26)</td>
<td>27.5 (28)</td>
<td>0.001</td>
</tr>
<tr>
<td>34-36 wk</td>
<td>31 (14)</td>
<td>31 (6)</td>
<td>0.717</td>
</tr>
</tbody>
</table>

## Table 2: Genes related to Mortality in VIP KO mice (N=5 WT mice and N=5 KO mice)

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>KO/WT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 1a</td>
<td>Il1a</td>
<td>1.69</td>
<td>0.037</td>
</tr>
<tr>
<td>Interleukin 1b</td>
<td>Il1b</td>
<td>1.76</td>
<td>0.046</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>Il6</td>
<td>1.42</td>
<td>0.006</td>
</tr>
<tr>
<td>TNF beta</td>
<td>Tnfsf13b</td>
<td>0.74</td>
<td>0.005</td>
</tr>
<tr>
<td>TNF alpha</td>
<td>Tnf alpha</td>
<td>1.02</td>
<td>0.860</td>
</tr>
<tr>
<td>Interferon gamma</td>
<td>Ifng</td>
<td>1.13</td>
<td>0.367</td>
</tr>
<tr>
<td>Interferon gamma receptor 1</td>
<td>Ifngr1</td>
<td>0.78</td>
<td>0.010</td>
</tr>
<tr>
<td>Leptin</td>
<td>Lep</td>
<td>2.82</td>
<td>0.029</td>
</tr>
<tr>
<td>Leptin receptor</td>
<td>Lepr</td>
<td>0.57</td>
<td>0.030</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

![KO/WT](https://www.researchpub.org/journal/jcvd/jcvd.html)
The resulting phenotype in VIP KO mice is that the clinical features of asthma, pulmonary hypertension and heart failure confer reduced survival, with significantly increased mortality beginning at 5 months and completely rendering the cohort by 20 months, compared to no mortality in the WT group. WT mice do not have the clinical phenotype of asthma, pulmonary hypertension and heart failure and are also heavier with higher normal core temperatures.

Thus, VIP has critical roles in modulating the immune system since its absence raises susceptibility to lethality. The lack of VIP in VIP knockout mice in this context explains the reduced survival, lower body weight, lower core temperature, since a combination of upregulated genes and previously reported cytokines in conjunction with progressive right ventricular hypertrophy may play critical roles in the health of these mice. Targeting these pathways may provide insight into models of obesity, premature death, cancer-related cachexia, primary pulmonary arterial hypertension, severe asthma, premature aging and thermoregulation.

As with humans who have pulmonary arterial hypertension, our VIP KO mice died of their disease. While patients in a recent cohort were hospitalized and treated with medications so that right ventricular failure was the sole cause of death in less than half, our VIP KO mice were untreated, so we were able to document the relentless progression of right ventricular hypertrophy and associated increased death rate. Pulmonary arterial hypertension is an orphan disease with estimated prevalence of 19.5 per 100,000 persons. VIP may have a novel role in protecting against death in patients with pulmonary arterial hypertension with progressive right ventricular hypertrophy.

V. CONCLUSIONS

VIP has been shown to be a master regulator in mammalian biology, orchestrating homeostasis and survival in mice. Lack of the VIP gene, and subsequent loss of VIP expression, is associated with premature death, progressive age-related right ventricular hypertrophy as a surrogate of pulmonary hypertension, lower body weight and hypothermia. Inasmuch as humans also have VIP distributed throughout the body: mast cells, nerve terminals, the hippocampus of the brain, airways; these studies support the concept of VIP as a novel therapy in pulmonary hypertension and heart failure. Suppressor of Cytokine Signaling KO mice have been associated with suppressed TNF-α and IFN-γ and greater associated cardiac dysfunction. Hence, in VIP KO mice the elevation of these cytokines may be a counter-regulatory mechanism to offset the lower weight from illness related to pulmonary hypertension and lower temperature from these cytokines.

VI. ACKNOWLEDGMENTS

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REFERENCES


Anthony M. Szema, M.D., earned his BS in industrial & management engineering from Rensselaer Polytechnic Institute, Troy, NY, in 1986, and an MD from Albany Medical College, Albany, NY, in 1991. He completed his internship in medicine at Johns Hopkins Bayview, Baltimore, MD, 1991-2, residency in internal medicine at Hahnemann University Hospital, Philadelphia, PA, 1992-4, and fellowships in pulmonary diseases, critical care medicine, and clinical adult and pediatric allergy/immunology at NY/Presbyterian Hospital-Columbia Campus, 1994-6, studying CD40 Ligand in the laboratory of Michael Yellin and Leonard Chess, Division of Rheumatology/Immunology.

Dr. Szema is past recipient of an NIH K08 award with his mentor Distinguished SUNY Professor Sami I. Said, M.D., to study vasoactive intestinal peptide (VIP) knockout mice with asthma and pulmonary hypertension, and is currently a co-investigator on an NIH R21 award studying the effects of Hurricane Sandy on World Trade Center Rescue Workers with asthma (PI Adam Gonzalez, PhD). Dr. Szema is Assistant Professor of Medicine and Surgery, Stony Brook University School of Medicine, Stony Brook, NY; Chief, Allergy Section, Veterans Affairs Medical Center, Northport, NY; and Managing Member of Three Village Allergy & Asthma, PLLC, South Setauket, NY.

Sayyed A. Hamidi, MD, received the MD degree from Shiraz University, Iran. He has been a Research Assistant Professor of Medicine at Stony Brook University School of Medicine since 2005. Dr. Hamidi begins a residency in internal medicine at the Bronx Veterans Affairs Medical Center, Bronx, NY, in July, 2014. He is past recipient of a Gilead Scholars Award and has been a co-investigator on several NIH awards with mentor Sami I. Said, MD prior to Dr. Said’s death last year.