

## Isothiocyanates Ameliorate the Symptom of Heart Dysfunction and Mortality in a Murine AIDS Model by Inhibiting Apoptosis in the Left Ventricle

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**ABSTRACT** Cardiac involvement has been reported in as many as 45–55% of patients with human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), and significant cardiac morbidity is reported in 6–7% of HIV patients. We investigated the inhibitory effects of isothiocyanates (ITCs) on heart dysfunction and mortality by regulating apoptosis in the left ventricle of the heart in a murine AIDS model. Mice were divided into six groups: an uninfected group, an untreated LP-BM5 retrovirus-infected group, and four LP-BM5 retrovirus-infected groups treated with one of four ITCs (sulforaphane [SUL], indolo[3,2-*b*]carbazole, benzyl isothiocyanate [BITC], or phenethyl isothiocyanate [PEITC]). After 16 weeks, the median survival time of the LP-BM5 retrovirus-infected mice was 87 days, whereas that of the uninfected control group and all ITC treatment groups was over 112 days. SUL, PEITC, and BITC significantly inhibited apoptosis in the left ventricle by increasing the Bcl-2/Bax ratio compared with LP-BM5-infected mice. In addition, SUL and PEITC suppressed inducible nitric oxide synthase (iNOS) expression at both the mRNA and protein levels in the left ventricle of heart tissue infected with the LP-BM5 retrovirus by inactivating cytoplasmic nuclear factor  $\kappa$ B (NF- $\kappa$ B). In conclusion, LP-BM5 retrovirus infection was related to survival of murine AIDS mice, and NF- $\kappa$ B-mediated iNOS expression may be an important mediator of left ventricle dysfunction of the heart. Furthermore, certain ITCs may have the potential to improve AIDS-related heart dysfunction due to their inhibition of apoptosis by decreasing iNOS and Bax expression through suppression of NF- $\kappa$ B.

**KEY WORDS:** • apoptosis • inducible nitric oxide synthase • isothiocyanates • murine AIDS

### INTRODUCTION

**H**UMAN IMMUNODEFICIENCY VIRUS (HIV) infection is the fourth leading cause of death worldwide.<sup>1</sup> An estimated 2.5 million new HIV infections and 2.1 million acquired immune deficiency syndrome (AIDS) deaths occurred in 2007, according to the United Nations Joint Program on HIV/AIDS, and almost 33.2 million adults and children worldwide are currently living with HIV/AIDS.<sup>2,3</sup> With >7000 new infections each day, the HIV pandemic remains an important public health issue.<sup>4</sup>

HIV infection and AIDS have a well-recognized association with myocarditis and dilated cardiomyopathy.<sup>5</sup> In particular, HIV-related heart muscle disease can present as a dilated cardiomyopathy, as isolated left ventricular dysfunction, or as nonspecific right heart changes.<sup>6</sup> Cardiac

involvement has been reported in as many as 45–55% of patients with HIV infection and AIDS, and significant cardiac morbidity from HIV disease is estimated to be 6–7% and mortality is estimated to be 1–6%.<sup>7,8</sup> Dilated cardiomyopathy, myocarditis, endocarditis, pericardial disease, and pulmonary hypertension remain major cardiac manifestations of HIV disease.<sup>7,9</sup>

Two classes of the most abundant naturally occurring dietary chemopreventive compounds are polyphenolic and isothiocyanate (ITC)-containing compounds, which have completely different chemical structures. Polyphenolic compounds are characterized by phenolic functional groups, whereas ITC-containing compounds are characterized by the sulfur-containing N=C=S functional group.<sup>10</sup> ITCs are synthesized and stored in cruciferous vegetables as glucosinolates. Allyl ITC from cabbage, mustard, and horseradish, benzyl ITC (BITC), phenethyl ITC (PEITC) from watercress and garden cress, and sulforaphane (SUL) from broccoli, cauliflower, brassicas, and kale are commonly used ITC-containing compounds.<sup>10,11</sup> Their biological activities are strongly related to modulation of cellular redox status, and

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numerous studies have documented their antioxidant properties. Furthermore, they have also been shown to inhibit cancer growth and metastasis in animal carcinogenesis models as well as cell culture models and to induce the apoptosis of cancer cells.<sup>11,12</sup>

For the past two decades, most therapeutic research in the field of HIV/AIDS has focused on the development of vaccines and antiretrovirals to block specific steps in the virus life cycle. Antiretroviral therapy has been extremely effective at controlling viral replication in HIV-infected individuals.<sup>4</sup> Highly active antiretroviral therapy is currently widely used, which may have resulted in the changes in the prevalence of antiretroviral drug resistance in drug-naïve patients.<sup>13,14</sup>

Various natural products without any experimental evidence of anti-HIV activities, such as garlic, ginseng, and shiitake mushrooms, are being used by AIDS patients in some countries.<sup>15,16</sup> Therefore, in this study, we investigated the inhibitory effects of indolo[3,2-*b*]carbazole (ICZ), BITC, PEITC, and SUL from cruciferous vegetables on apoptosis induced by overexpression of inducible nitric oxide (NO) synthase (iNOS) and induction of Bax in the heart left ventricle of a murine AIDS (MAIDS) model. The present study is the first to demonstrate that ITCs have inhibitory effects on AIDS-related heart dysfunction.

## MATERIALS AND METHODS

### *Animals and treatment*

Female C57BL/6 mice, 4 weeks old, were obtained from Charles River Laboratories (Wilmington, DE, USA). They were housed in transparent plastic cages with stainless wire lids (three or four mice per cage) in the animal facility of Kyung Hee University (Yongin, Korea). Our animal use protocol was approved by the Institutional Animal Care and Use Committee of Kyung Hee University. The housing facility was maintained at 20–22°C and 60–80% relative humidity with a 12-h light/dark cycle. For the study, mice were randomly assigned to the uninfected control group or infected groups that received various ICTs by oral administration. Oral administration of treatments began at 2 days postinfection with the LP-BM5 murine leukemia retrovirus and continued for 16 weeks for a survival test (24 mice per group) and 12 weeks for immunological analysis (10 mice per group). All mice were fed the AIN-93M maintenance diet during the experiments.

ITCs including SUL, BITC, ICZ, and PEITC were purchased from Sigma (St. Louis, MO, USA). Female C57BL/6 mice were administered one of the ITCs or vehicle once daily by oral gavage at a dose of 1 mM/kg in 100  $\mu$ L of ethanol per day. A dose of 1 mM/kg was chosen as this dose has been shown to be effective against several types of cancer in a previous study.<sup>17</sup> LP-BM5 murine leukemia retrovirus (generously donated from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA) was administered intraperitoneally in 0.1 mL of minimum essential medium with an esotropic titer of 4.5 log<sub>10</sub> plaque-forming units  $\times$  10<sup>-3</sup>/L; this titer induces disease with

a time course comparable with that previously published.<sup>18</sup> Infection of female C57BL/6 mice with LP-BM5 retrovirus results in the rapid induction of clinical symptoms with virtually no latent phase. The mice were infected 2 days prior to initiation of oral administration of ITCs. The infection and treatment period for immunological analysis was 12 weeks for all groups. When MAIDS developed, all mice were sacrificed by ethyl ether anesthesia. Hearts were rapidly excised, and the left ventricle was separated for TdT-mediated dUTP nick-end labeling (TUNEL) assay and histological analysis. Heart left ventricle tissue for real-time polymerase chain reaction (PCR) and western blot was frozen in liquid nitrogen and stored at -70°C until analysis.

### *Survival test*

Female C57BL/6 mice, 4 weeks old, were randomly assigned to one of six groups (24 mice per group) and fed one of the ITCs or vehicle for 16 weeks to assess the effects of ITCs intake on survival. Mice were monitored daily for survival and conditions, while their drinking water and AIN-93M diet were changed every 3 days. Median survival time is expressed as the number of days until 50% of mice in the group had died.

### *TUNEL assay*

The degree of apoptosis was evaluated by the TUNEL method. Paraffin blocks containing left ventricle tissues were cut in 6- $\mu$ m-thick sections, and the sections were stained for the detection of apoptosis using the TUNEL apoptosis detection kit (GenScript, Piscataway, NJ, USA). In brief, the sections were deparaffinized and rehydrated, incubated with blocking solution (3% H<sub>2</sub>O<sub>2</sub> in methanol) for 10 min, and permeabilized in a solution containing 0.1% Triton X-100 and 0.1% sodium citrate in water. A TUNEL reaction mixture containing fluorescein ITC-12-dUTP was applied to the fixed cells, and apoptotic cells were determined under a fluorescence microscope with an excitation wavelength of 450 nm and an emission wavelength of 565 nm.

### *Real-time PCR*

Heart left ventricle tissue was disrupted and homogenized with rotor-stator homogenizers in the presence of RLT buffer (lysis buffer) (Qiagen, Valencia, CA, USA) with  $\beta$ -mercaptoethanol until a completely homogeneous lysate was obtained. Total RNA was isolated from left ventricle tissue lysate using an RNeasy<sup>®</sup> Mini kit (Qiagen). One microgram of total RNA obtained from the left ventricle tissues was reverse-transcribed using reverse-transcript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) to produce cDNAs. Real-time PCR was performed using the selective primer sets with Universal SYBR<sup>®</sup> Green PCR Master Mix according to the manufacturer's instruction (Qiagen). The primer sequences used for amplification of the sense and antisense strands were as follows: GAPDH, 5'-CCATGAGAAGTATGACAACAGCC-3' and 5'-TGGCAG

GTTTTTCTAGACGG-3'; iNOS, 5'-TCTTGGGTCTCCGC TTCTCGTC-3' and 5'-TGGCTGGTACATGGGCACAGA G-3'; Bcl-2, 5'-TTCCAGCCTGAGAGCAACCCAATG-3' and 5'-TGACCCACCGAACTCAAAGAAGG-3'; and Bax, 5'-AGGATGATTGCTGACGTGGACACG-3' and 5'-AAG ATGGTAACTGTCTGCCATGTGG-3'. Data analyses were carried out using a 7500 System with SDS software version 1.3.1 (Applied Biosystems Inc., Foster City, CA, USA).

#### Histological analysis

Detection of iNOS was performed by immunostaining. Heart left ventricles were fixed in 4% formaldehyde. Following dehydration and embedding in paraffin by routine protocol, heart left ventricle tissue sections (6  $\mu$ m) were deparaffinized, rehydrated, and blocked with 1.5% normal horse serum. Expression of iNOS was detected using anti-iNOS (diluted 1:200) and Vectastain<sup>®</sup> avidin-biotin peroxidase and biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were visualized under a microscope.

#### Western blot analysis

Heart left ventricle tissues were lysed using RIPA buffer containing 50 mM phosphate buffer (pH 7.4), 0.5% Nonidet P-40, 0.1% sodium dodecyl sulfate, and a protease inhibitor cocktail (Sigma). Equal amounts of protein were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electrotransferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). Membranes were blocked with 5% nonfat dry milk at 4°C for 1 h and then incubated with anti-nuclear factor  $\kappa$ B (NF- $\kappa$ B), inhibitory  $\kappa$ B (I $\kappa$ B), and iNOS antibodies (Cell Signaling, Beverly, MA, USA) overnight at 4°C. After washing and binding with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h, the reaction products were visualized with chemiluminescent reagents (Amersham, Piscataway).

#### Statistics

All experiments were presented as mean  $\pm$  SD values. The significance of treatment effects was assessed by Duncan's multiple range tests after one-way analysis of variance using SAS (Cary, NC, USA) software. Test statistics were considered significant at the  $P < .05$  level.

## RESULTS AND DISCUSSION

#### Effect of ITCs on the survival of LP-BM5 retrovirus-infected mice

In the MAIDS model, the pathogenesis of LP-BM5 viral infection is characterized by immune dysfunction with many changes similar to those reported in human HIV infection. It is well established that LP-BM5 viral infection provokes an enlargement of lymphoid organs accompanied by systemic imbalance of T helper cytokines and dysfunction of immune cells.<sup>19</sup> During recent de-

ades, many clinicians have suggested that significant cardiac dysfunction is an important cause of AIDS-related mortality that was estimated to be 6–7%.<sup>7</sup> In fact, many people with HIV infection generally have depressed left ventricular function as measured by either fractional shortening or contractility that is associated with cardiomyopathy, resulting in the increased incidence of mortality.<sup>20</sup> Although few studies have estimated the life expectancy of HIV-infected people with cardiomyopathy, it has been well accepted that antiviral treatment against HIV improves the symptoms of heart dysfunction, suggesting that proper treatment for those people could increase the life expectancy. In animal studies, two murine leukemia virus models have been advocated to study HIV: the MAIDS model and a temperature-sensitive virus (*ts1*) model. The MAIDS model, which involves the LP-B5 virus, has been widely used as a model comparable to HIV.<sup>21,22</sup> The LP-BM5 retrovirus-induced MAIDS model has some similarities with HIV-induced AIDS in humans.<sup>23</sup> To examine the protective effect of ITCs in LP-BM5 retrovirus-infected mice, we determined mortality in the uninfected and infected groups. As shown in Table 1, the median survival time of the infected control mice was 87 days, whereas the median survival time of the uninfected control and all ITC-treated mice was over 112 days. Death from profound immunodeficiency usually occurred within 16–24 weeks after LP-BM5 retrovirus infection.<sup>22</sup> In this study, viral activity of LP-BM5 might be more virulent because it was injected directly without frozen storage. Therefore, the median survival time of the infected control mice was shorter than expected based on other studies. The importance of cardiac dysfunction is demonstrated by the reported median survival to AIDS-related death of 101 days in patients with left ventricular dysfunction versus 472 days in patients with normal hearts as analyzed by echocardiography at a similar infection stage.<sup>24</sup> The increased survival rate achieved by oral administration of ITCs indicates that ITCs have protective effects against LP-BM5 retrovirus infection-induced immunodeficiency.

TABLE 1. MEDIAN SURVIVAL TIME OF MICE IN THE STUDY OF LP-BM5 RETROVIRUS INFECTION AND ISOTHIOCYANATE ADMINISTRATION

LP-BM5 retrovirus/ treatment	Median survival time (days) <sup>a</sup>	Number of mice (%) <sup>b</sup>
-/-	>112	16 (100)
+/-	87	5 (31.3)
+ICZ	>112	12 (75)
+BITC	>112	16 (100)
+PEITC	>112	16 (100)
+SUL	>112	16 (100)

<sup>a</sup>Median survival time was represented by the interval at which 50% death in the treatment group occurred.

<sup>b</sup>Number of mice surviving after 16 weeks (%).

BITC, benzyl isothiocyanate; ICZ, indolo[3,2-*b*]carbazole; PEITC, phenethyl isothiocyanate; SUL, sulforaphane.



*Effect of ITCs on apoptosis of heart left ventricle tissue cells in LP-BM5 retrovirus-infected mice*

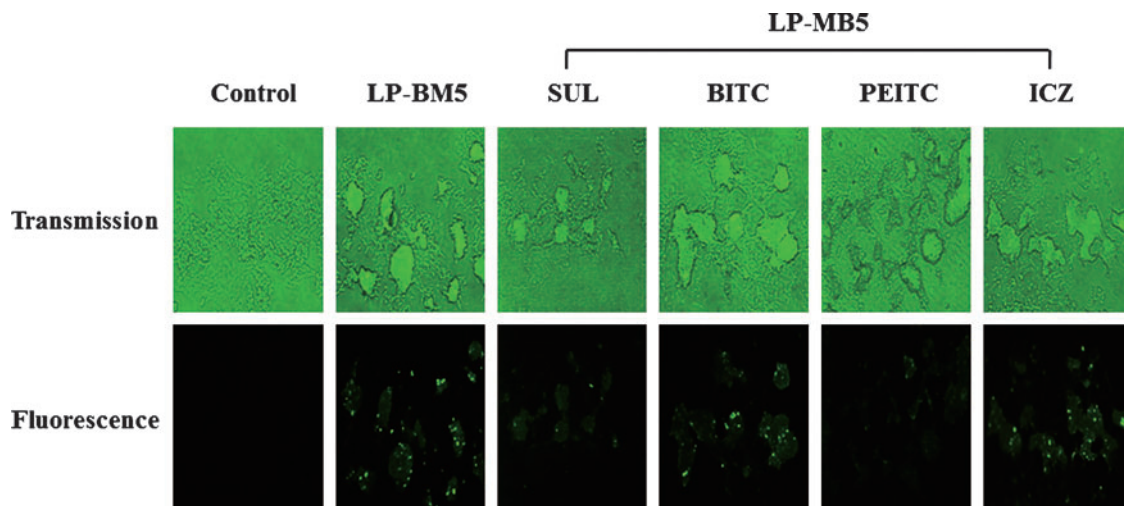
Apoptosis or programmed cell death is a major form of cell death characterized by stereotypic morphological and biochemical features including plasma membrane blebbing and shrinkage, chromosomal DNA fragmentation, and chromatin condensation.<sup>25,26</sup> Aberrant regulation of apoptosis has been observed in many serious disorders, including neuronal disease, AIDS, autoimmune diseases, and cancer.<sup>27</sup> To examine whether the protective effect of ITCs were mediated by the inhibition of apoptosis in LP-BM5 retrovirus-infected mice, we performed the TUNEL assay. As shown in Figure 1, the number of TUNEL-positive or apoptotic cells increased significantly in LP-BM5 retrovirus-infected mice compared with uninfected control mice. The number of TUNEL-positive cells was significantly lower in the SUL- and PEITC-treated groups compared with the LP-BM5 retrovirus-infected control group. These results indicate that oral administration of ITCs inhibited apoptosis in heart left ventricle tissues of LP-BM5 retrovirus-infected mice. However, there might be an argument against the function of ITCs because those have been long used as apoptotic reagents in cancer studies. It may be suggested that, because cancer cells are known to be more susceptible to certain chemical reagents, ITCs show different effects on apoptosis depending upon the types of tissues or organs. In addition, unlike in cancer cells, ITCs could regulate the HIV replication that ameliorates the symptoms of HIV-related diseases.<sup>28</sup> Cardiomyocyte apoptosis underlies many forms of heart disease, including viral cardiomyopathy. Inducers of cardiomyocyte apoptosis include myocardial cell stretch, pressure overload, oxygen radicals, NO, cytokines such as tumor necrosis factor- $\alpha$ , and viruses.<sup>29</sup> Pozzan *et al.*<sup>24</sup> detected cardiomyocyte apoptosis in the isolated cells of 10 of 23 patients (43.5%) with AIDS. Cardiomyocyte apoptosis may also contribute to HIV-related heart muscle failure.<sup>24</sup>

*Effect of ITCs on mRNA expression of Bcl-2 and Bax in the heart left ventricles of LP-BM5 retrovirus-infected mice*

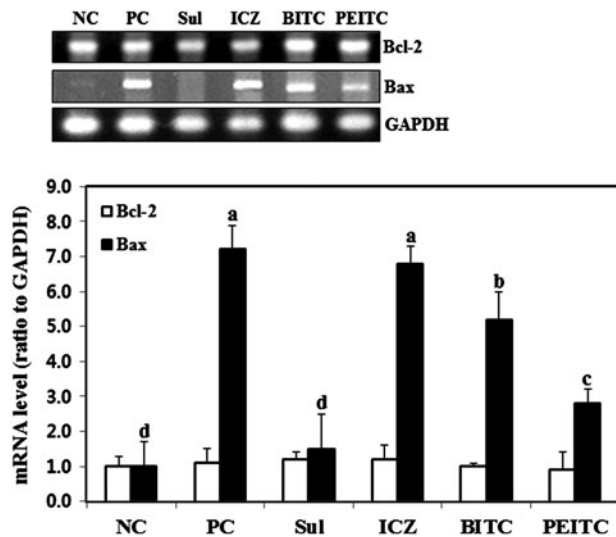
An imbalance in the expression of Bcl-2 family proteins in mitochondrial membranes, specifically anti-apoptotic Bcl-2 and pro-apoptotic Bax, leads to cytochrome *c*-mediated apoptosis.<sup>30</sup> Therefore, to further explore the effects of the ITCs on apoptosis, we measured the expression of Bcl-2 and Bax by western blotting. There were no significant differences in Bcl-2 expression between groups, whereas Bax expression was significantly higher (sevenfold) in the LP-BM5 retrovirus-infected control group than in the uninfected control group (Fig. 2). This increased Bax/Bcl-2 ratio probably triggered apoptosis in the LP-BM5 retrovirus-infected control mice. There were no significant changes in the mRNA expression of Bcl-2 after oral administration of ITCs. However, ITCs decreased the mRNA levels of Bax significantly, thereby reducing the Bax/Bcl-2 ratio. In HIV infection, oxidative stress leads to apoptosis by increased expression of pro-apoptotic genes, such as those coding for caspase-2, caspase-3, caspase-9, and Bax.<sup>31–33</sup> According to several studies, ITCs possess potent antioxidative capacity against oxidative stress, including menadione, *tert*-butyl hydroperoxide, 4-hydroxynonenal, and peroxyxynitrite.<sup>34,35</sup> Taken together, these results suggested that inhibition of apoptosis by oral administration of ITCs, at least in part, is caused by decreasing Bax expression via antioxidative effects of ITCs.

*iNOS expression in the heart left ventricles of LP-BM5 retrovirus-infected mice*

NO is one of the most important regulatory factors of the cardiovascular system. NO is synthesized from L-arginine by NO synthases, including neuronal type I synthase, endothelial type III synthase, and inducible type II synthase

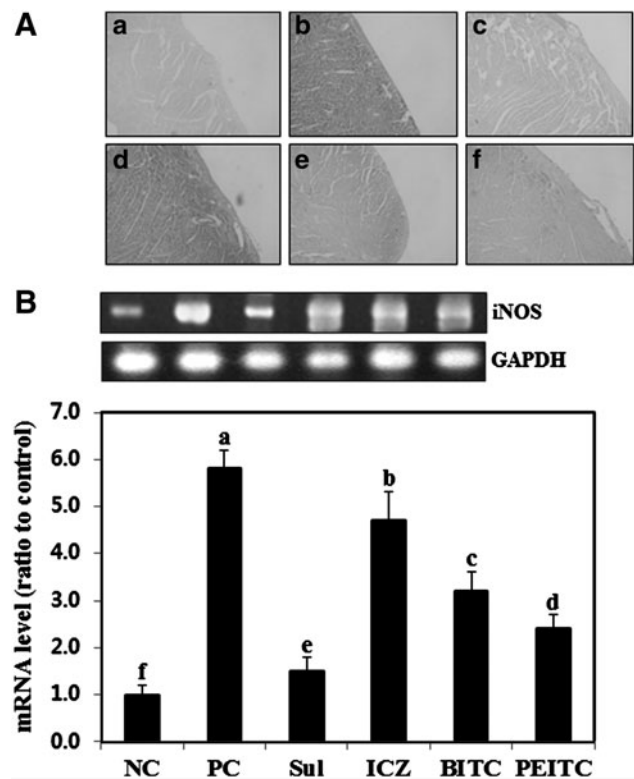


**FIG. 1.** Inhibitory effects of isothiocyanates on apoptosis in heart left ventricle tissues of LP-BM5 retrovirus-infected C57BL/6 mice. Apoptosis of left ventricle tissues was measured by TdT-mediated dUTP nick-end labeling assay, as described in Materials and Methods. Color images available online at [www.liebertpub.com/jmf](http://www.liebertpub.com/jmf)



**FIG. 2.** Effect of isothiocyanates on mRNA expression of Bcl-2 and Bax in the left ventricles of C57BL/6 mice infected with the LP-BM5 retrovirus: uninfected control (negative control [NC]), infected control (positive control [PC]), SUL, ICZ, BITC, and PEITC. Left ventricle tissues were prepared and used for real-time polymerase chain reaction, as described in Materials and Methods. Data are mean  $\pm$  SD values of at least three independent experiments, each performed in triplicate. <sup>abcd</sup>Different letters indicate a significant difference ( $P < .05$ ) as determined by Duncan's multiple range test. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

(iNOS), a family of isoenzymes with distinct functional, biological, and regulatory properties.<sup>36,37</sup> The overexpression of specific cardiac iNOS results in cardiac fibrosis, dilation, and premature death.<sup>38</sup> Barbaro *et al.*<sup>39</sup> reported that iNOS staining intensity was higher in patients with HIV-associated cardiomyopathy than in patients with idiopathic dilated cardiomyopathy. In addition, Sam *et al.*<sup>40</sup> reported that heart left ventricular dysfunction and myocardial apoptosis were diminished in a mouse iNOS knockout model. Therefore, to examine the effects of ITCs on LP-BM5 retrovirus-induced apoptosis, we measured iNOS expression in the left ventricle of the heart of LP-BM5 retrovirus-infected control mice. The optical density of iNOS staining in LP-BM5 retrovirus-infected mice was significantly higher than in uninfected control mice (Fig. 3A), indicating that LP-BM5 induced iNOS expression in the left ventricle. It is interesting that SUL, PEITC, and BITC showed a diminished amount of brown color in left ventricle specimens, suggesting that long-term administration of those ITCs for LP-BM5 retrovirus-infected mice is, in part, able to inhibit the induction of iNOS in the left ventricle due to viral infection. However, there was no current evidence for ICZ administration on decreasing iNOS expression in LP-BM5 retrovirus-infected left ventricle. For more detailed measurements, as shown in Figure 3B, the level of iNOS mRNA was significantly increased (5.7-fold) in LP-BM5 retrovirus-infected mice compared with uninfected control mice. Expression of iNOS was significantly lower in mice that received oral administration of ITCs, compared with LP-BM5 retrovirus-infected control



**FIG. 3.** Inhibitory effect of isothiocyanates on inducible nitric oxide synthase (iNOS) and iNOS mRNA expression in the left ventricle tissues of C57BL/6 mice infected with the LP-BM5 retrovirus. Left ventricle tissues were prepared and used for (A) immunohistochemistry for tissues from mice treated with (a) NC, (b) PC, (c) SUL, (d) ICZ, (e) BITC, and (f) PEITC and (B) real-time polymerase chain reaction, as described in Materials and Methods. Data are mean  $\pm$  SD values of at least three independent experiments, each performed in triplicate. <sup>abcdef</sup>Different letters indicate a significant difference ( $P < .05$ ) as determined by Duncan's multiple range test.

mice. In particular, SUL and PEITC decreased iNOS mRNA levels significantly by 430% and 370%, respectively, compared with LP-BM5 retrovirus-infected control mice. These results are in agreement with a previous study that showed that iNOS immunoreactivity was stronger in the myocardial cells of HIV-infected patients with dilated cardiomyopathy compared with the myocardial cells of controls.<sup>39</sup>

#### *Suppressive effect of ITCs on NF- $\kappa$ B activation in the heart left ventricles of LP-BM5 retrovirus-infected mice*

NF- $\kappa$ B is present in all cells in a resting state in the cytoplasm. Only when it is activated and translocated to the nucleus is the usual sequence of events generated. Under resting conditions, NF- $\kappa$ B consists of a heterotrimer of p50, p65, and I- $\kappa$ B $\alpha$  in the cytoplasm. The phosphorylation, ubiquitination, and degradation of I- $\kappa$ B $\alpha$  lead to the release of the p50-p65 heterodimer, which then translocates to the nucleus, binds its specific 10-basepair consensus site, and regulates the genetic expression of key pro-inflammatory cytokines during progression of HIV infection.<sup>41</sup> It has been

well accepted that iNOS expression is induced by cytokine-induced transcription factors, such as interferon and NF- $\kappa$ B, which recognize response elements within the iNOS promoter.<sup>38,42</sup> NF- $\kappa$ B, a pro-inflammatory transcription factor, is involved in iNOS, cyclooxygenase-2, and tumor necrosis factor- $\alpha$  expression as well. As shown in Figure 4, the expression of iNOS was clearly increased by LP-BM5 retrovirus infection. Oral administration of SUL clearly decreased the expression of iNOS close to the level seen in uninfected controls. However, ICZ and BITC treatment did not result in a significant difference in iNOS expression compared with the LP-BM5 retrovirus-infected control. To determine the mechanism underlying the suppression of iNOS by ITCs, we analyzed the expression and translocation of NF- $\kappa$ B, which is known to regulate the expression of iNOS. Oral administration of each of the four ITCs after infection resulted in a dramatic reduction in the translocation of NF- $\kappa$ B. In particular, SUL and PEITC inhibited phosphorylated NF- $\kappa$ B to a greater extent than equal concentrations of ICZ and BITC. These results suggested that SUL and PEITC suppress iNOS induction significantly by inhibiting NF- $\kappa$ B translocation. Furthermore, NF- $\kappa$ B transcribes the mRNAs of several anti-apoptotic members of the Bcl-2 family. Because the expression of Bcl-2 and Bax is directly activated by NF- $\kappa$ B,<sup>43,44</sup> we speculated that LP-BM5 retrovirus infection would increase the expression of NF- $\kappa$ B and that ITCs would suppress this response. When these findings are taken together, SUL and PEITC dramati-

cally suppressed apoptosis by reducing Bax mRNA levels through the inhibition of NF- $\kappa$ B.

In conclusion, oral administration of SUL and PEITC significantly inhibited apoptosis in heart left ventricle tissues of LP-BM5 retrovirus-infected mice by reducing iNOS expression, the Bax/Bcl-2 ratio, and the NF- $\kappa$ B signaling pathway. These results suggest that ITCs are potential therapeutic candidates for treatment of AIDS-related heart dysfunction.

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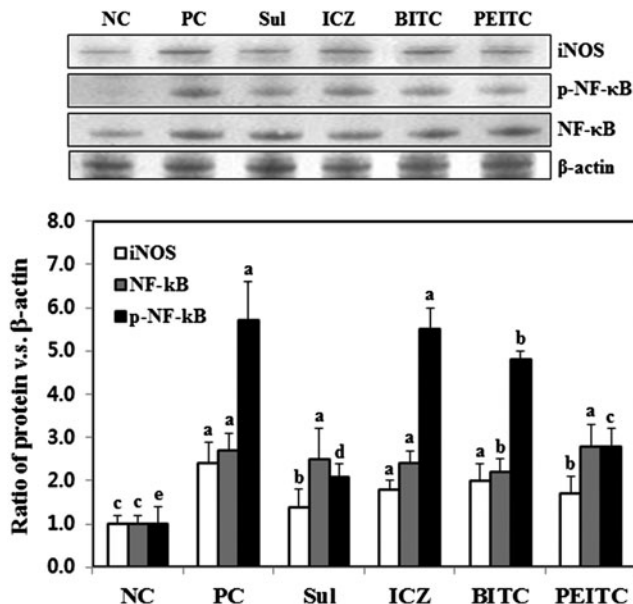
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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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**FIG. 4.** Inhibitory effect of isothiocyanates on overexpression of iNOS and activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) in the left ventricle tissues of C57BL/6 mice infected with the LP-BM5 retrovirus. Left ventricle tissues were prepared and used for western blot, as described in Materials and Methods. Data are mean  $\pm$  SD values of at least three independent experiments, each performed in triplicate. <sup>abcde</sup>Different letters indicate a significant difference ( $P < .05$ ) as determined by Duncan's multiple range test. p-NF- $\kappa$ B, phosphorylated NF- $\kappa$ B.



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