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Management of the Virulent Influenza Virus Infection by Oral Formulation of Nonhydrolized Carnosine and Isopeptide of Carnosine Attenuating Proinflammatory Cytokine-Induced Nitric Oxide Production

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Inducible nitric oxide synthase (iNOS) plays an important role in mediating inflammation. In our studies, we found that iNOS-derived NO was significantly increased in the serum samples of 150 patients infected with influenza A virus in comparison with samples of 140 healthy individuals. In human lung epithelial cells, infection with influenza A virus or stimulation with poly(I:C) + interferon-gamma resulted in increased mRNA and protein levels of both interleukin-32 and iNOS, with subsequent release of NO. Activated macrophages are also a source of nitric oxide (NO), which is largely produced by iNOS in response to proinflammatory cytokines. In this review article, the presented findings have many important implications for understanding the Influenza A (H1N1) viral pathogenesis, prevention, and treatment. The direct viral cytotoxicity (referred cytopathic effect) is only a fraction of several types of events induced by virus infection. Nitric oxide and oxygen free radicals such as superoxide anion ($O_2^{-\cdot}$) are generated markedly in influenza A (including H1N1) virus–infected host boosts, and these molecular species are identified as the potent pathogenic agents. The mutual interaction of NO with $O_2^{-\cdot}$ resulting in formation of peroxynitrite is operative in the pathogenic mechanism of influenza virus pneumonia. The toxicity and reactivity of oxygen radicals, generated in excessive amounts mediate the overreaction of the host's immune response against the organs or tissues in which viruses are replicating, and this may explain the mechanism of tissue injuries observed in influenza virus infection of various types. The authors revealed the protection that carnosine and its bioavailable nonhydrolized forms provide against peroxynitrite damage and other types of viral injuries in which immunologic interactions are usually involved. Carnosine (beta-alanyl-L-histidine) shows the pharmacologic intracellular correction of NO release which might be one of the important factors of natural immunity in controlling the initial stages of influenza A virus infection (inhibition of virus replication) and virus-induced regulation of cytokine gene expression. The protective effects of orally applied nonhydrolized formulated species of carnosine include at least direct interaction with nitric oxide, inhibition of cytotoxic NO-induced proinflammatory condition, and attenuation of the effects of cytokines and chemokines that can exert profound effects on inflammatory cells. These data are consistent with the hypothesis that natural products, such as chicken soup and chicken breast extracts rich in carnosine and its derivative anserine (beta-alanyl-1-methyl-L-histidine) could contribute to the pathogenesis and prevention of influenza virus infections and cold but have a limitation due to susceptibility to enzymatic hydrolysis of dipeptides with serum carnosinase and urine excretion after oral ingestion of a commercial chicken extract. The developed and patented by the authors formulations of nonhydrolized in digestive tract and blood natural carnosine peptide and isopeptide (gamma-glutamyl-carnosine) products have a promise in the Influenza A (H1N1) virus infection disease control and prevention.

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INTRODUCTION

> The United States is disturbingly underprepared to deal with either a massive outbreak of the flu or a sudden shortage of vaccine, and we cannot hope to skate by any longer.
>
> —Larry Craig

World Health Organization: 42 countries report swine flu cases

Despite vaccines and antiviral substances influenza still causes significant morbidity and mortality world wide. Better understanding of the molecular mechanisms of influenza virus replication, pathogenesis and host immune responses is required for the development of more efficient means of prevention and treatment of influenza. Influenza A virus (H1N1), which replicates in epithelial cells and leukocytes, regulates host cell transcriptional and translational systems and activates and downregulates apoptotic pathways.

The human swine flu outbreak continues to grow in the United States and internationally (Figures 1A–D). Today, United States Centers for Disease Control and Prevention (CDC) reports additional cases of confirmed swine influenza and a number of hospitalizations of swine flu patients. Internationally, the situation is more serious too, with additional countries reporting confirmed cases of swine flu. In response to the intensifying outbreak, the World Health Organization (WHO) initially raised the worldwide pandemic alert level to phase 4. A phase 4 alert is characterized by confirmed person-to-person spread of a new influenza virus able to cause "community-level" outbreaks. The increase in the pandemic alert phase indicates that the likelihood of a pandemic has increased. The WHO has pushed its influenza pandemic alert to the second highest level and instructed all countries to immediately activate their pandemic preparedness plan. Clinicians should consider the possibility of swine influenza virus infections in patients presenting with febrile respiratory illness.

CDC is issuing updated interim guidance daily in response to the rapidly evolving situation. This includes updated interim guidance for clinicians on how to identify and care for people who are sick with novel H1N1 flu now that more widespread illness has been detected in the United States. CDC recommends that testing and antiviral treatment be prioritized for those with severe respiratory illness and those at highest risk of complications from seasonal influenza. This includes children younger than 5 years old, pregnant women, people with chronic medical conditions and weakened immune systems, and people 65 years and older. In addition, CDC has provided information for the public on what to do if they develop flu-like symptoms. There are antiviral medicines one can take to prevent or treat swine flu. There is no vaccine available right now to protect against swine flu. One can help prevent the spread of germs that cause respiratory illnesses like influenza by

- Covering your nose and mouth with a tissue when you cough or sneeze. Throw the tissue in the trash after you use it.
- Washing your hands often with soap and water, especially after you cough or sneeze. You can also use alcohol-based hand cleaners.
- Avoiding touching your eyes, nose or mouth. Germs spread this way.
- Trying to avoid close contact with sick people.
- Staying home from work or school if you are sick.

The 1918 Spanish flu epidemic was caused by an influenza A (H1N1) virus, killing more than 500,000 people in the United States, and up to 50 million worldwide. The possible source was a newly emerged virus from a swine or an avian host of a mutated H1N1 virus. Many people died within the first few days after infection, and others died of complications later. Nearly half of those who died were young, healthy adults. Influenza A (H1N1) viruses still circulate today after being introduced again into the human population in the 1970s.

The new strain is an apparent reassortment of several strains of influenza A virus subtype H1N1, which analysis at the United States CDC identified as a strain endemic in humans, a strain endemic in birds, and 2 strains endemic in American and Eurasian pigs (swine).[1]

Drug targeting analysis of swine flu virus infection disease reveals diverse therapeutic platforms

Antiviral drugs may be an important tool in controlling early events in the emergence of new

FIGURE 1. A, The ongoing outbreak of novel influenza A (H1N1) continues to expand in the United States and internationally. CDC expects that more cases, more hospitalizations and more deaths from this outbreak will occur over the coming days and weeks. The swine flu virus is spread in exactly the same way as ordinary colds and flu. CDC continues to take aggressive action to respond to an expanding outbreak caused by novel H1N1 flu. CDC's response goals are to: (1) reduce transmission and illness severity, and (2) provide information to help health care providers, public health officials and the public address the challenges posed by this emergency. Symptoms of swine flu in people are similar to the symptoms of regular human flu and include fever, cough, sore throat, body aches, headache, chills and fatigue. B, Swine influenza virus, like other animal flu viruses, usually causes a respiratory illness confined to the animal population that serves as its source. The swine flu virus can be spread to humans who are in close contact with pig populations. Modes of human-to-human transmission are similar to those of seasonal flu viruses. Collecting a respiratory specimen and sending it to the CDC can confirm a suspected diagnosis of swine flu. The virus that has been isolated in Mexico and the US is being described as a new subtype of A/H1N1 not previously detected in pigs or humans. While the cases in the United States have been mild with only one requiring hospitalization, the CDC earlier declared a public health emergency over concern of a potential swine flu pandemic. Symptoms of swine flu are similar to those of seasonal flu and include fever, malaise, lethargy, cough, anorexia, and in many cases nausea, vomiting, and diarrhea. Prevention of transmission is also similar to seasonal flu. C, Judy Trunnell, a 33-year-old school teacher who had just given birth to a healthy baby girl. A Texas woman with the new H1N1 swine flu died earlier this April 2009 week, the second death attributed to the virus on U.S. soil, Texas Health officials said on Tuesday. It was the first time that a U.S. resident succumbed to the virus, after a toddler from Mexico City who had crossed into the United States to visit family in the border town of Brownsville died at a Houston hospital on April 27, 2009. The Texas Department of State Health Services said a woman with chronic underlying health conditions died in April 2009 in Cameron County, which is located on the U.S.–Mexico border and includes Brownsville. The United States now has 403 confirmed cases of the new H1N1 flu, in 38 states, the U.S. Centers for Disease Control and Prevention said. D, Symptoms of H1N1 Influenza of a typical patient are similar to the regular human flu and include in this clinical case: fever temperature $>100°$F, chills, cough, runny nose, sore throat, head and body aches, fatigue, nausea, vomiting, diarrhea. People with the flu can infect others up to 24 hours before they know that they are sick and they may continue to spread the flu 7 or more days after they recover from the flu.

subtypes in the human population. Two types of drugs that target influenza are licensed amantadine and inhibitors of the neuraminidase enzyme. Amantadine is unhelpful for the current outbreak because the strains involved already harbor a mutation, making them resistant to the drug. Neuraminidase inhibitors are active against the avian types of N protein but are not stockpiled in any quantity appropriate for mass use. Governments may need to mobilize funding to establish stockpiles.

With cases of swine flu increasing around the world, many countries are investing in large stockpiles of a single drug, Tamiflu (oseltamivir). But the strategy could be problematic. Influenza viruses can become resistant to antiviral drugs. The widespread use of a single drug is likely to increase the risk that a resistant strain will emerge. If such a strain were to spread widely, the effectiveness of antiviral drugs in treating infected patients, and their ability to slow the spread of a pandemic, would be greatly reduced. In

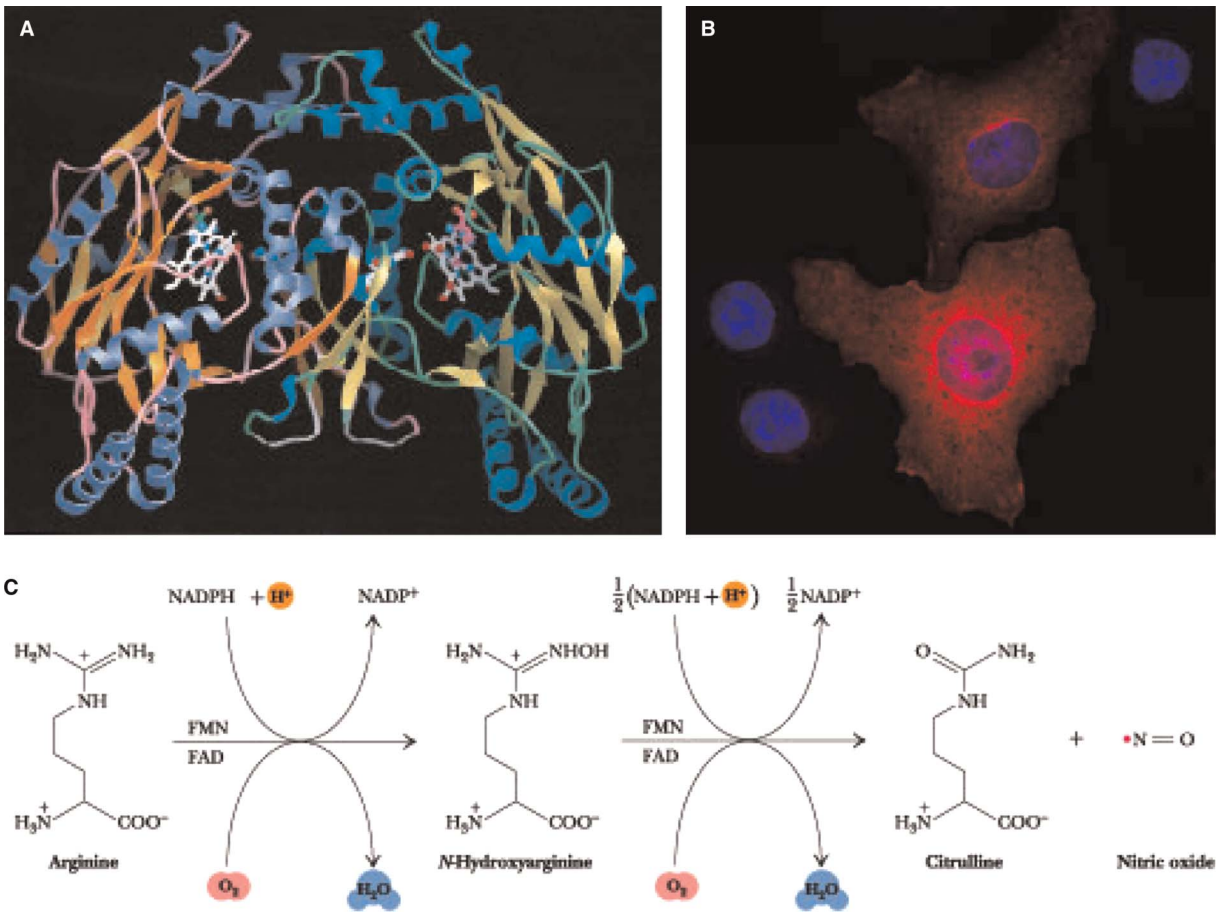

FIGURE 2. The synthesis of NO by NOS. NO· is synthesized from arginine by NOS in 2 consecutive monooxygenase reactions . As a dissolved gas, NO is capable of rapid diffusion across membranes in the absence of any apparent carrier mechanism. This property makes NO· a particularly attractive second messenger because NO· generated in one cell can exert its effects quickly in many neighboring cells. NO·has a very short cellular half-life (1–5 seconds) and is rapidly degraded by noenzymatic pathways. The NO synthases identified in the brain and in the linings of blood vessels produce small amounts of NO·for signaling purposes. Another form of the enzyme, found in macrophages is termed inducible NOS or iNOS. This latter form of enzyme is critical for the immune response, but it is also implicated in a variety of diseases that involve overproduction of NO·, including septic shock, Alzheimer's disease, multiple sclerosis, stroke, inflammatory bowel disease, rheumatoid arthritis, and many forms of inflammation. A, The structure of the oxygenase domain of iNOS, is shown in the dimeric form. The monomer structure is elongated and curved, with an unusual single-domain α-β fold that resembles a left-handed baseball catcher's mitt. The essential heme is held in the mitt's webbed β-sheet palm with its distal face directed to a large cavity. B, iNOS expression during the macrophage oxidative burst triggered by bacterial endotoxin or virus infection.

a global influenza pandemic, small stockpiles of a secondary flu medication—if used early in local outbreaks—could extend the effectiveness of primary drug stockpiles.

The previously recommended influenza vaccines for the southern and northern hemispheres, including that for the 2009/2010 flu season, are ineffective against the new strain.[2] Current development, large-scale manufacturing, distribution, and delivery of a new vaccine takes several months[2] though the first doses could be ready as early as June 2009.[3] The WHO Director-General announced that production of the unchanged seasonal vaccine should continue for now, and that the WHO would assist the development process for an effective vaccine.[4] U.S.-based medical product company Baxter International has requested a virus sample from the WHO to begin development of a new vaccine.[5] Baxter has patented a cell-based technology that may allow the company to develop a vaccine in half the time it usually takes, possibly cutting development time from 6 months to 3.[6]

An alternative to vaccination used in the 1918 flu pandemic was the direct transfusion of blood, plasma, or serum from recovered patients. Though medical

experiments of the era lacked some procedural refinements, 8 publications from 1918 to 1925 reported that the treatment could approximately halve the mortality in hospitalized severe cases with an average case-fatality rate of 37% when untreated.[7,8] Of the available antiviral treatments for influenza, the WHO stated that the viruses obtained from the human cases with swine influenza in the United States were sensitive to oseltamivr (Tamiflu)[9] and zanamivir (Relenza) but resistant to amantadine and rimantadine.[10] Tamiflu and Relenza also have a preventative effect against influenza virus A).[11]

As the outbreak develops over the ensuing days and weeks it should become clear whether this virus will spread worldwide. The danger signs will be seeing human to human transmission with any noteworthy frequency, and genetic changes becoming apparent in viruses isolated from infected people. Even in the event of yet another lucky escape, more measures must be taken to limit the amplification of viruses with pandemic potential in the wet markets around the world.[12]

Human disease associated with the H5N1 "bird flu" outbreak in 1997 was unusually severe,[13,14] for reasons that have remained unclear. H5N1/97 viruses have been shown to induce the overproduction of proinflammatory cytokines[15] and to evade their antiviral effects.[16] The reemergence of H5N1 viruses has been reported that are pathogenic for both humans and waterfowl and show that their genotypes are related to their proinflammatory cytokine induction phenotypes.[17] Influenza A virus infection causes respiratory disease by mechanisms which are still largely obscure.

The pathogenetic Influenza A (H1N1) virus challenges: involvement of nitric oxide and cytokines release

Virus infection induces numerous characteristic responses in cells that prevent productive infection, viral replication, and virion production. The expression of proinflammatory and antiviral genes by infected cells contributes to the inhibition of virus replication, although the mechanisms of transcriptional regulation are incompletely understood. Macrophages represent an integral part of innate immunity based on their ability to generate robust inflammatory and antiviral responses. The production and release of cytokines and inflammatory mediators such as interleukin-1 (IL-1) and cytotoxic molecules such as nitric oxide contribute to the suppression of virus replication. This is particularly evident given the observation that the IFN-mediated inhibition of virus replication is dependent upon nitric oxide.[18]

The limited cytopathic effect of the virus on epithelial cells lining the respiratory tract cannot itself explain the pneumonia resulting from infection. There is increasing evidence from mouse models of influenza that suggests that tissue damage is linked to the host's effector mechanisms, which are activated by and directed against the invading virus.[19,20] Several investigators have established the importance of cytotoxic T cells in the development of pneumonia.[21,22] However, the biochemical nature of this self-inflicted damage remains to be established. Several groups of authors have suggested that effector mechanisms involved in the clearance of pathogens, such as the generation of reactive oxygen species (ROS) by phagocytes, could participate in the development of the disease.[23,24] ROS could contribute to the tissue damage seen in lungs either directly by oxidizing lipids, proteins and nucleic acids[25] or indirectly by activating certain proteases.[26] Influenza viruses and paramyxoviruses can directly activate monocytes and polymorphonuclear leukocytes in vitro to generate ROS.[23]

Nitric oxide (NO), initially identified as an endothelium-derived relaxing factor,[27] is synthesized from L-arginine by NO synthase (NOS) (Figure 2) in numerous mammalian cells and tissues.[28] To date, at least 3 major categories of NOS have been clarified: the constituent and calcium-dependent isoforms are principally present in endothelial and neuronal cells. The remaining one is the inducible and calcium-independent isoform (inducible nitric oxide synthase, iNOS), which has been demonstrated in a wide variety of cells such as macrophages,[29–31] smooth muscle cells,[32] endothelial cells,[33] hepatocytes,[34] and cardiac myocytes.[35] It has been shown that iNOS produces a high amount of NO, which is sustained for a long period, when the cells are activated by various stimuli as lipopolysaccharide (LPS), IL-1, and tumor necrosis factor (TNF).[36] The nanomolar concentrations of NO

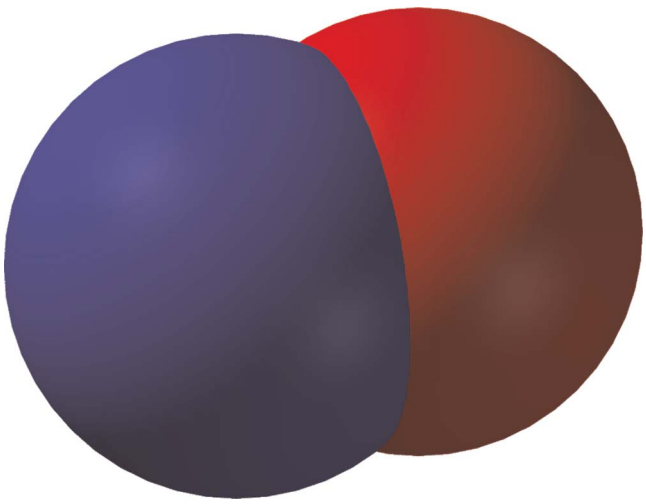

FIGURE 3. Space-filling model of nitric oxide.

are considered sufficient for intracellular signaling, and especially, NO produced via iNOS in macrophages acts as a defense molecule against bacteria and parasites (Figure 2).[37] Recently, studies on the role of NO production have expanded to virus infections. Indeed, a beneficial effect of iNOS-mediated NO production has been demonstrated in several virus infections such as herpes simplex virus, vesicular stomatitis virus, Japanese encephalitis virus, and vaccinia virus infections.[18,38–47]

In the case of influenza virus infection, a dual role of NO (Figure 3) has been shown. An in vitro study reported an inhibitory effect of NO on virus growth,[48] and another reported that NO played a pathologic role in the influenza virus–induced pneumonia in mice.[49] The results have shown clearly that excessive NO biosynthesis is induced in mouse lung via production of interferon-γ (IFN-γ) by influenza virus infection.

Overproduction of NO was substantiated by determining an increase in both NOS activity and expression of iNOS mRNA in virus-infected lung and by means of electron spin resonance (ESR) spectroscopy.[49] However, adding hemoglobin, a strong NO-binding protein and thus an inactivator of NO activity, did not reverse the *N*-methyl-D-aspartate induced inhibition of viral production, suggesting that NO might exert its antiviral effects inside the NO-producing cells.[39] Considering the dual role (beneficial and detrimental roles) of NO on certain inflammatory disorders and virus infections, the inductive activity of influenza virus on the iNOS-mediated NO production independent on its infectivity might contribute to a modification of influenza virus infection.[50]

Although the mode of action of NO is not fully understood, it has been shown that NO can affect the function of iron- and thiol-containing proteins.[29,51,52] such as guanylate cyclase, ribonucleotide reductase, aconitase, and mitochondrial electron transport enzymes.[53] iNOS can be induced in a number of different cell types by cytokines or bacterial products. It has been demonstrated that iNOS is expressed in murine macrophages,[54] mouse T cells,[55] human hepatocytes,[56,57] alveolar macrophages,[58–62] and mononuclear cells.[63,64] NO can also be released from human airway epithelial cells, the primary target for influenza viruses, after stimulation with gamma interferon, IL-1β, and TNF-α.[65–67] Interestingly, the production of these cytokines is induced shortly after infection with influenza viruses.[68–71] Furthermore, it has been demonstrated that the expression of the hemagglutinin of influenza viruses can activate nuclear factor-κB, a transcription factor which has been shown to regulate the expression of a number of cytokine genes, including the TNF-α and IL-1β genes.[72,73]

Taken together, the data demonstrate that NO inhibits the replication of influenza viruses, probably during the early steps of the virus replication cycle, involving the synthesis of vRNA and mRNA encoding viral proteins.[48] Therefore we hypothesize that the production of NO by iNOS in airway epithelial cells, induced by cytokines which are known to be synthesized shortly after infection with influenza viruses by NK cells and macrophages,[68,70] provides an antiviral effect in these cells. This mechanism would reduce primary replication of influenza viruses before other effector mechanisms of the immune system, such as those mediated by B and T lymphocytes, are activated to control the infection. To be beneficial for the host, the production of NO must be tightly regulated to exert antiviral rather than harmful effects, such as cell death and tissue destruction. This regulation of NO production could be at the transcriptional and the translational level but also at the level of enzyme activity during infection, as was demonstrated in a mouse *Cryptococcus neoformans* model.[74] At present, it is unclear whether the level of in vivo synthesized NO compares with the level of NO released from NO donors in vitro. Only recently, limited information has become available on the induction of NO after respiratory virus infections, including influenza.[53,75] The contribution of NO to the pathogenesis of influenza virus–induced pneumonia of mice[49,76] was also observed in Herpes Simplex Virus Type 1-induced pneumonia in this species.[77] However, a clear antiviral effect of NO was also demonstrated against the latter virus in vitro.[18,40] Although the metabolic effects of NO may contribute to its antiviral activity, this mechanism also is likely to account for some of the antiviral properties of other host response mediators such as interferon. Cytostasis is a well-recognized effect of NO and was observed with each of the cell lines employed in the provided studies.[78,79] One of the most thoroughly studied effects of NO is its ability to activate the heme-containing enzyme, guanylate cyclase.[80,81]

Interaction of NO with the cytosolic form of guanylate cyclase stimulates the production of cGMP which, in turn, mediates many well-characterized intracellular events.[80–84] Little is known about the effect of cGMP on virus replication.

The experiments showed that cytokine-induced high-output NO·synthesis from L-arginine is not restricted to cells specialized for host defense, such as macrophages, but is potentially an activity of most somatic cells involved in cell-mediated immune reactions.[85] This expanded the scope of innate resistance and cellular immunity by showing that somatic cells not specialized for host defense have the potential to participate in innate resistance and cell-mediated immunity via cytokine-induced high-output NO·synthesis. The

studies also revealed a novel mechanism for cytokine-induced NO·mediated autotoxicity (ie, high-output NO·synthesis by nonmacrophage somatic cells).

The significance of cytokine-induced nitric oxide synthesis during innate resistance and cell-mediated immune reactions is important. High-output NO·synthesis appeared to be a component of innate resistance and cell-mediated immunity, but it also appeared to have the potential for producing autotoxicity if not properly regulated.

These are formidable but not insurmountable challenges to future advances in understanding the role of cytokine-induced high output NO· synthesis in humans. It is likely that NO·, because of its inherent chemical versatility, will function in many ways and on many levels in response to the influenza A virus infection disease and stress-producing situations in intact biologic systems, mammals including humans.

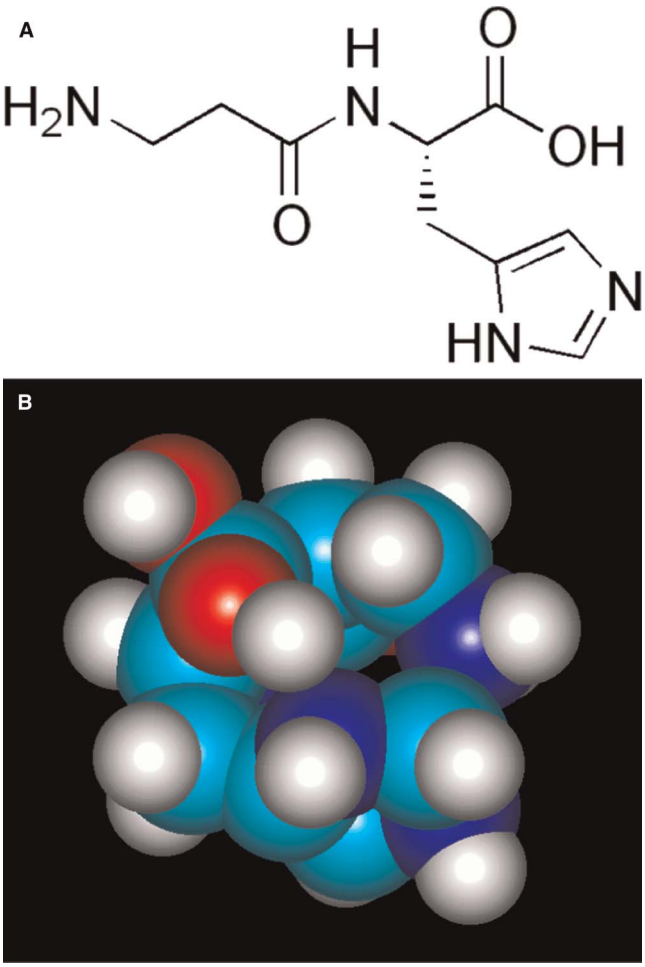

FIGURE 4. Structure of L-carnosine dipeptide shown as chemical structure (A) and energy-minimized structure (space filling model) (B).

Nitric oxide involves in carnosine metabolism: the role in cell biochemistry and function

Carnosine (β-alanyl-L-histidine) and related compounds are natural constituents of excitable tissues possessing diverse biological activities.[86,87] The level of carnosine in tissues is controlled by a number of enzymes transforming carnosine into other carnosine related compounds, such as carcinine, *N*-acetylcarnosine, anserine or ophidine (by decarboxylation, acetylation, or methylation, respectively) or its cleavage into the amino acids, histidine and β-alanine. Hydrolysis is mainly due to tissue carnosinase (EC 3.4.13.3), which is widely distributed among different subjects[88,89] or serum carnosinase (EC 3.4.13.20), obtained in brain and blood plasma of primates and humans.[90,91]

Carnosine acts as a physiologic buffer, a metal ion chelator, a free radical scavenger, and finally as an antioxidant (Figures 4A and B).[92,93] Besides the known anti-aging properties of this dipeptide, it has been demonstrated that carnosine plays a role in inflammation. In fact, carnosine-inhibited hydrogen peroxide induced IL-8 release.[94] IL-6 and TNF-α were reduced by the oral administration of carnosine in an animal model of diabetes,[95] and finally carnosine proved to decrease the secretion of transforming growth factor-β (TGF-β) and of various extracellular matrix components induced by high doses of glucose in vitro.[96]

Carnosine has been proven to scavenge ROS and alpha-beta unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress.[97–99] It can oppose glycation[100,101] and it can chelate divalent metal ions. The important studies have produced clinical and experimental evidence of beneficial effects of N-acetylcarnosine in treating cataracts of the eyes, these and other ophthalmologic benefits have been proven.[102–109] Overall, these low molecular mass antioxidant peptidomimetics add significantly to the defense provided by the enzymes superoxide dismutase, catalase, and glutathione peroxidases.[110,111]

Furthermore, carnosine has been shown to counteract peroxynitrite-dependent protein alterations.[112] Peroxynitrite is a well-known highly toxic oxidizing and nitrating agent that is produced in vivo by the spontaneous reaction of superoxide anions ($O_2^{-\cdot}$) with nitric oxide (NO·). For this reason NO is implicated in many physiologic and pathologic processes,[113–125] depending on the amount produced and the type of cell or tissue involved.

The results[112] showed that the carnosine-like dipeptides efficiently protect tyrosine against nitration, alpha 1-antiproteinase against inactivation and

human low-density lipoprotein against modification by peroxynitrite. Carnosine exerts its protective effect at concentrations similar to those found in human tissues. In addition, some synthetic pseudodipeptides, structurally related to carnosine but stable to hydrolytic enzymes, possess protective properties against peroxynitrite-dependent damage similar to the natural dipeptides. These pseudodipeptides may represent stable mimics of the biologically active carnosine suitable for pharmacological applications.

In the respect of various aspects of carnosine metabolism, it is particularly relevant to take into account the different roles of nitric oxide synthase (EC 1.14.13.39) isoforms.[117] As stated above, nitric oxide has now been shown to be derived from L-arginine in macrophages, endothelial cells, and possibly other cell types. Its physiologic role in macrophages may be as a cytotoxic agent. However, nitric oxide produced by endothelial cells is thought to trigger vascular smooth muscle relaxation through activation of the enzyme guanylate cyclase.[117] NO produced by neuronal nitric oxide synthase (nNOS or NOS-1) and endothelial NOS (eNOS or NOS-3) is a second messenger that triggers signal transduction through the soluble guanylyl cyclase (sGC)/3′,5′-cyclic guanosine monophosphate (cGMP) pathway to exert its physiologic functions (ie, neurotransmission and vasodilatation); conversely, the great amount of NO produced by inducible NOS (iNOS or NOS-2) in macrophages and glial cells plays an antimicrobial role during host defense. However, iNOS overactivation is also a typical hallmark of many pathological conditions where it is responsible for increased free-radical insults. In addition, impaired NO production is associated with many disorders, especially in the central nervous system, such as stroke, migraine, and neurodegenerative processes.[117,118]

It recently has been shown that carnosine inhibits NO-dependent activation of guanylate cyclase,[119] which is attributed to NO interaction with the Fe-heme prosthetic group, ruling out that carnosine could interact directly with nitric oxide. Recently, the evidence has appeared that carnosine has an aptitude for interacting with nitric oxide.[120] The authors studied the ability of carnosine to interact with NO, both in a cell-free system by competitive spectrophotometric and electrospray mass spectrometry (ESI-MS) analyses and in primary astroglial cell cultures treated with LPS and IFN-γ, a method widely used to induce iNOS and to promote neurotoxic conditions.[121] The authors revealed the evidence of carnosine interaction with NO and its protective effects against oxidative stress.

Non-hydrolized carnosine signaling pathways in relation to the molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression

Carnosine and anserine, the bioactive peptides found in most meats and fish, were tested for their ability to modulate neutrophil and U937 cell function, specifically with respect to respiratory burst, IL-1β production and apoptosis. Both peptides increased the respiratory burst and interleukin-1 beta production of human neutrophils but not of U937 cells. They suppressed apoptosis of human neutrophils but enhanced apoptosis of U937 cells as assessed by DNA strand breaks. These results suggest that carnosine and anserine have the capacity to modulate the immune response at least in human neutrophils.[122] In carnosine-stimulated macrophages the activity of membrane 5′-AMP nucleotidase decreases on days 1–3 after injection which points to alleviation of adenosine-induced inhibition and to macrophage activation.

Carnosine increases the cytostatic and phagocytotic activities of macrophage coupled to $O_2^{-\cdot}$ production. The mechanism of the stimulating effect of carnosine on macrophages seems to consist in the dipeptide interaction with specific receptors localized on the plasma membrane of macrophageal cells.[123]

Luminol-dependent chemiluminescence of the phorbol myristate acetate (PMA)-stimulated human neutrophils decrease more than by 50% in the presence of physiologic concentrations of carnosine (20 mM). This inhibition is the result of carnosine ability to scavenge hypochlorite (OCl-), because carnosine exerts a similar effect on chemiluminescence produced by myeloperoxidase-H2O2-Cl- and OCl(−)-H2O2 systems. The previously undocumented property of this dipeptide to scavenge active oxygen species requires further experiments.[124] Dipeptide carnosine and amino acid taurine have been found to actively interact with hypochlorite anion. Chloramine complexes obtained during this reaction were more stable in case of taurine.[125]

The observed general decrease in the antioxidant buffering capacity may reduce the ability of tissues to protect against potential oxidative stress. Such stress can occur during bacterial superinfections, which are common in influenza, thereby rendering the host more susceptible to the pathogenic effects of such agents. In addition, ROS produced in the lung may inactivate protease inhibitors, resulting in increased protease activity. Using an in vitro system consisting of alpha 1-antiprotease, trypsin and HOCl as the oxidant, a group of authors has shown that the infectivity of influenza viruses can be increased up to 10,000-fold by proteolytic cleavage of haemagglutinin,

leading to activation of the fusogenic properties of this protein.[126]

In this review article, we demonstrate the pathogenic role of NO (Figure 3) in influenza virus–induced pneumonia in conjunction with generation of superoxide.

Oxygen-free radicals such as superoxide anion ($O_2^{-\cdot}$) were generated markedly in influenza virus–infected mouse lung, and these molecular species were identified as the potent pathogenic agents. This finding has many important implications for understanding viral pathogenesis: namely, the direct viral cytotoxicity (referred cytopathic effect) is only a fraction of several types of events induced by virus infection. The toxicity and reactivity of oxygen radicals, which are presumably generated in excessive amounts by the overreaction of the host's immune response against the organs or tissues in which viruses are replicating, may explain the mechanism of tissue injuries observed not only in influenza virus infection in mice, but also in other types of viral diseases in which immunologic interactions are usually involved.[127]

IFN-γ has been implicated as the major cytokine responsible for NOS-2 induction during influenza A-induced pneumonitis.[49] IFN-γ thus seems to cooperate with immune components otherwise subject to suppression by NOS-2 and not by NOS1 and/or NOS3. Complete inhibition of NOS-2 by disruption of the gene encoding it, rather than the partial inhibition afforded by treatment of NOS-$2^{+}/^{+}$ mice with N^{ω}-methyl-L-arginine (L-NMA), also appeared necessary to permit expression of the IFN-γ–dependent anti-influenza mechanism.[76]

The pathogenicity of influenza virus infection involves, at least in part, overreaction of the immune responses of the host rather than a direct effect of virus multiplication. Xanthine oxidase, which is responsible for the generation of oxygen-free radicals, was elevated in serum and lung tissue of mice infected with influenza virus. To test the theory that oxygen-free radicals are involved in pathogenesis, free radicals were removed by injecting superoxide dismutase (SOD), a specific superoxide radical scavenger, which was conjugated with a pyran copolymer. The conjugate protected mice against a potentially lethal influenza virus infection if administered 5–8 days after infection. These findings indicate that oxygen radicals are important in the pathogenesis of influenza virus infection, and that a polymer-conjugated SOD has therapeutic potential for this virus infection and other diseases associated with free radicals.[128]

Superoxide reacts with nitric oxide to generate highly reactive metabolites such as peroxynitrite. This compound is able to oxidize proteins, resulting in direct nitration of tyrosine residues. Protein structure and function can be subsequently altered and enzymatic activity affected. Proteins containing nitrotyrosine residues have been detected in different pathologies associated with enhanced oxidative stress and increased levels of peroxynitrite.[129] In the reported study[129] immunohistochemistry with a specific anti-nitrotyrosine antibody showed intense staining of alveolar phagocytic cells such as macrophages and neutrophils and of intraalveolar exudate in the influenza virus–infected lung. In these experiments provided in mice, influenza virus pneumonia was produced with influenza virus A/Kumamoto/Y5/67(H2N2).[49] These results strongly suggest formation of peroxynitrite in the lung through the reaction of NO with $O_2^{-\cdot}$, which is generated by alveolar phagocytic cells and xanthine oxidase. In addition, administration of N^{ω}-monomethyl-L-arginine (L-NMMA) resulted in significant improvement in the survival rate of virus-infected mice without appreciable suppression of their antiviral defenses. On the basis of these data, the authors concluded that NO together with $O_2^{-\cdot}$ which forms more reactive peroxynitrite may be the most important pathogenic factors in influenza virus-induced pneumonia in mice.[49]

Overproduction of NO seems to be directly related to hypotension and shock observed in endotoxemia and sepsis.[130] Furthermore, involvement of NO has been suggested in the pathogenesis of inflammatory disorders [eg, immune complex alveolitis[131] and arthritis[132] in rats], in the pathogenesis of neurodegenerative disease,[133] and in the neuropathogenesis of some neurotropic virus infections.[134–136] As described previously, a large gap is found in the time course of influenza virus pneumonia in mice between the peak time of virus propagation in the lung and the maximum lethal effect, which correlates well with the extent of pathological change in the pneumonic lung,[127,137] namely, a direct cause (viral replication) and effect (pathological change) is not observed in influenza virus-induced pneumonia in mice. This indicates that pulmonary injury can be attributed to a noninfectious mechanism rather than the cytopathic effect depending on viral replication per se. Complicated interactions between virus and host are reported for many viral diseases, and immunologic effects of the host on propagation of the virus are known to be involved in pathogenesis of some viral diseases.[127,128,137,138] Furthermore, taking into consideration the previous findings suggesting NO-mediated neutrotoxicity in virus-induced encephalopathy,[134–136,139] it is highly possible that mutual interaction of NO with $O_2^{-\cdot}$ is operative in the pathogenic mechanism of influenza virus pneumonia in mice. Thus removal of NO and/or $O_2^{-\cdot}$ to prevent formation of peroxynitrite may be beneficial to the infected hosts.

Carnosine protection against peroxynitrite damage is particularly relevant to the above described findings, and also by now there has been obtained an evidence of direct interaction with nitric oxide.[120] In the cited study[120] the authors revealed the protection that carnosine provides against nitric oxide (NO)-induced cell death in primary rat astroglial cell cultures treated with LPS and IFN-γ, a well-known neurotoxic proinflammatory condition. A correlation was found between cell protection and NO free-radical scavenging activity of carnosine. By competitive spectrophotometric measurement and electrospray mass spectrometry analysis in cell-free experiments, the authors demonstrated a direct interaction of the dipeptide with NO. A comparison of carnosine with its homologues or derivatives (homocarnosine and carcinine) and with its amino acid constituents (L-histidine and beta-alanine) highlighted that only histidine showed significant scavenging activity.

Other studies have revealed carnosine-induced upregulation of stress protein expression and nitric oxide synthesis, both of which may stimulate proteasomal elimination of altered proteins.[140] Whereas previously there has been evidence to indicate that arginine is the natural substrate for generating nitric oxide synthase (NOS) activity, it is now shown that carnosine, which is widely distributed in tissues, is likely to be the true substrate. In tissue sections it gave a stronger NOS reaction than does arginine.[141] The virulent avian influenza virus A/Ty/Ont/7732/66 (H5N9) (Ty/Ont) causes a rapid destruction of lymphoid cells in infected birds.[142] Avian macrophage cell lines, HD11 and MQ-NCSU, support productive replication of Ty/Ont and other influenza viruses. Therefore, the ability of these cell lines to produce nitric oxide (NO), a potentially cytotoxic mediator, in response to infection with Ty/Ont is an important molecular mechanism controlling virus replication. Although treatment with bacterial LPS resulted in high NO levels, infection of macrophages with Ty/Ont resulted in NO levels lower than NO levels in untreated cells. Furthermore, Ty/Ont was able to inhibit the positive response to LPS in cultures simultaneously treated with LPS and virus. However, inactivated influenza virus did not exhibit this inhibitory effect. Different strains of influenza virus varied in their ability to inhibit NO production by the macrophages; this may be related to the level of virus replication in these cells.[134] These data suggest that the ability of the avian macrophage to activate the NO synthesis pathway is seriously impaired by infection with virulent influenza viruses such as Ty/Ont.

Pharmacological administration of carnosine may regulate protective virus replication function and/or immune cell protection against influenza virus infection thereby attenuating intracellular NO generation via NOS in macrophages without altering iNOS expression. The separate study reports that carnosine-induced hyperactivity may be linked to the constitutive NOS, rather than iNOS, in the brain. Central carnosine may regulate brain function and/or behaviors by NO generation via constitutive NOS in chicks.[143]

Immunohistochemical analysis of lung sections obtained from bleomycin-treated mice revealed a positive staining for iNOS in macrophages and neutrophils present in the alveolar space and in septal walls.[144] Carnosine treatment abolished immunostaining for iNOS in lungs of animals treated with bleomycin.

Nitric oxide mediates vaso- and bronchodilatation, and it is synthesized from L-arginine by two constitutive forms of NOS, which are involved in the physiological regulation of airway function.[145] However, iNOS generates much larger quantities of nitric oxide than the constitutive isoforms, and it is directly involved in host defense from infections[146] and in various models of inflammation.[147,148] Both the enzyme activity of NO synthase (NOS) and mRNA expression of the inducible NOS were greatly increased in the mouse lungs; increases were mediated by IFN-γ.[49] Excessive production of NO in the virus-infected lung was studied further by using ESR spectroscopy. In vivo spin trapping with dithiocarbamate-iron complexes indicated that a significant amount of NO was generated in the virus-infected lung. Furthermore, an NO-hemoglobin ESR signal appeared in the virus-infected lung, and formation of NO-hemoglobin was significantly increased by treatment with superoxide dismutase and was inhibited by L-NMMA administration.[49] Exogenous nitric oxide is able to stimulate in vitro fibroblast proliferation,[149] whereas iNOS upregulation in lung fibroblasts is associated with the early proliferative response to cytokine stimulation.[150] Finally, the pharmacologic inhibition and the genetic disruption of iNOS have been shown to reduce the development of inflammatory responses and fibrosis in lung of bleomycin-treated animals.[151]

Several groups of authors previously shown that oxygen radicals such as superoxide anion ($O_2^{-\cdot}$) are primary pathogenic molecules in influenza virus-induced pneumonia in mice.[127,128,137] Possible involvement of $O_2^{-\cdot}$ was also reported in cytomegalovirus infection in mice.[138]

It is now well known that iNOS can be induced in vascular smooth muscle cells, bronchial epithelial cells, and murine macrophages after stimulation with proinflammatory cytokines such as IFN-γ and TNF-α, LPS, and bacteria.[65,152] It is highly plausible, therefore, that NO could be produced in the mammal's lung after

infection with influenza virus. From another hand, carnosine shows direct NO-trapping ability and as such may be a valuable multifunctional molecule in the pharmacologic correction of NO, which might be one of the important factors of natural immunity in controlling the initial stages of influenza A virus infection and virus-induced regulation of cytokine gene expression.

Many relevant functions have been proposed for L-carnosine and related imidazole-containing compounds, including wound healing promoter, ion-chelant agent, antioxidant, and free-radical scavenger.[153] Carnosine prevents cellular toxicity in vitro, with a direct antiperoxidative activity on proteins,[154] lipids,[155] and DNA bases.[156] The antioxidant and metal ion-chelator properties of carnosine have been successfully tested on animal models of stomatitis and duodenal and gastric ulcers and on different ocular disorders.[102–111,157] Furthermore, carnosine has been proven to affect inflammation directly by modulating cytokine release. In an animal model of diabetes, carnosine reduced IL-6 and TNF-α,[87] whereas TGF-β and extracellular matrix deposition were reduced by carnosine after stimulation with high doses of glucose in vitro.[96] In the recent study, the authors have shown a significant reduction of tissue damage and cellular apoptosis in lungs of bleomycin-treated mice treated with carnosine.[144] Not only the extracellular matrix deposition evaluated histologically in lung sections of treated mice showed a reduced degree of fibrosis but also the alveolar architecture was preserved, indicating that the treatment with this antioxidant significantly prevented lung damage induced by bleomycin. The beneficial activity of carnosine administration after bleomycin treatment was reflected by some favorable clinical outcomes. Most notably, the beneficial effects given by this treatment resulted in the complete abrogation of the bleomycin-induced mortality.

Furthermore, carnosine proved efficacious to significantly lower total and biologically active TGF-β_1 levels. TGF-β_1 plays a central role in fibrotic disorders in different organs, including fibrosis of the lung. In fact, it stimulates collagen and fibronectin production in fibroblasts[158]; on the other hand, it can suppress the production of proteases that degrade the extracellular matrix.[159] TGF-β_1 has been shown to be increased in bleomycin-induced lung fibrosis in the alveolar inflammatory infiltrate.[160] Secretion of active TGF-β_1 by alveolar macrophages is augmented after bleomycin administration in mice, whereas latent TGF-β_1 secretion remains elevated for a prolonged length of time, and it is probable that the extent of inflammation and fibrosis in this model depend on the quantity of active TGF-β_1 available.[161] Finally, the increase of TGF-β_1 mRNA precedes the biosynthesis of type I and type III procollagen in lung fibrosis.[160] Lung edema and fall of body weight were virtually absent, and inflammation was significantly reduced in carnosine-treated animals.

Leukocytes recruited into the tissue can contribute to tissue destruction by the production of reactive oxygen metabolites, granule enzymes, and cytokines that further amplify the inflammatory response.[144]

Recently, carnosine and some carnosine derivatives have been shown to scavenge superoxide anion radicals[162] and to chelate copper (II), leading to a complex that shows SOD1-like activity with a catalytic constant equal to that found for native SOD1.[163,164]

Therefore, carnosine might be a valuable pharmacologic tool, limited only by its short half-life, which is a result of the cleavage activity of tissue carnosinase.[165] New carnosine derivatives that have similar biologic activity but greater stability (because they are not degraded by serum carnosinase) have recently been synthesized in our laboratory and are now under investigation as suitable agents for pharmacologic applications. Considering the presented results, carnosine may be considered a potential multifunctional drug with both chelating and antioxidant activity; these properties may prove useful for the treatment and the prevention of diseases in which ROS are thought to play a major role such as the interstitial pathologies of the lung.

Anti-flu effect of chicken breast extracts (CBEX) ("Jewish penicillin"). Chicken soup cure of influenza virus inflammation disease and cold may not be a myth. Bioavailable and bioactivated species of L-carnosine peptide

Great interest has been shown on a dietary intake of antioxidants, which can have a beneficial impact on the maintenance of good health. The hydroxyl, hypochlorite, and peroxynitrite radicals are typical ROS generated in human body. Previously, it has been found that hydrophobic botanical antioxidants exhibited specific antioxidant activity against hydroxyl radicals, whereas anserine and carnosine mixture, purified from chicken extract and vitamin C, exhibited antioxidant activities against hypochlorite (ClO) and peroxynitrite (ONOO) radicals, respectively. An antioxidant preparation of anserine-carnosine mixture, vitamin C, and ferulic acid prevented oxidative stress by ROS.[166]

Chicken extract is used commercially as a seasoning and is usually produced by concentration after extraction from chicken meat with hot water. CBEX is obtained via hot water extraction of chicken breast and contains among its primary constituents carnosine and anserine, which are histidine-containing

dipeptides present in the muscle tissues of most vertebrate species. Dietary intake of CBEX has been previously shown to buffer hydrogen ions formed during high-intensity exercise in human skeletal muscle cells, thereby inhibiting a decrease in muscle cell pH and subsequent muscle fatigue.[167] The objective of the recent study was to report the results of safety studies completed on CBEX. CBEX was determined to have an oral LD(50) value of more than 6000 mg/kg body weight in rats. Gavage doses of 500 or 2000 mg CBEX/kg body weight/day administered to rats for 90 days produced no toxicologically significant, dose-related, differences between control and treated animals with respect to body weight gain, food consumption, behavioral effects, hematologic and clinical chemistry parameters, absolute and relative organ weights, or gross and microscopic findings. In the presence or absence of metabolic activation, CBEX exerted no mutagenic activity in the Ames assay conducted in various strains of *Salmonella typhimurium* and *Escherichia coli*. The results of these investigations support the safety of CBEX enriched with histidine moieties as a potential dietary or therapeutic source of carnosine and anserine.[167] Carnosine-related dipeptides are rich in the commercially available supplement CBEX. To clarify the effects of CBEX on the brain, another group of authors examined whether single oral administration of CBEX (20 mL/kg) affects brain dipeptide and free amino acid concentrations in male Wistar rats.[168] CBEX significantly and time dependently increased carnosine and anserine levels in the plasma (at 120 minutes after injection, increase rates were 2976% and 4142%, respectively), hippocampus (64% and 78%), and hypothalamus (188% and 120%), but not in cerebral cortex. Significant and time-dependent increases in citrulline in the hippocampus (49%) and hypothalamus (41%) demonstrated generation of nitric oxide due to the increased carnosine and/or anserine levels in these brain regions. These findings suggest that CBEX modifies brain functions by increasing levels of these dipeptides.

The nasal route is rich with carnosine acting as the neurotransmitter. Chickens were intranasally inoculated with the swine influenza virus (SIV) A/swine/NC/307408/04 (H3N2) (NC/04 SIV) to determine the infectivity of a North American SIV for chickens, and the possibility of chicken meat serving as a transmission vehicle for SIV. White leghorn (WL) layer-type chickens were used for initial pathotyping and infectivity tests, and a more comprehensive intranasal pathogenesis study was done with white Plymouth rock (WPR) broiler-type chickens.

None of the NC/04 SIV-inoculated WL or WPR chickens displayed clinical signs. Serologic tests showed that the virus was able to infect both intranasally inoculated WL and WPR chickens, but the antibody titers were low, suggesting inefficient replication. Some of the NC/04 SIV-inoculated WL chickens shed low levels of virus, mostly from the alimentary tract, but viral shedding was not detected in NC/04 SIV-inoculated WPR chickens. The comprehensive pathogenesis study demonstrated that the virus did not cause systemic infections in WPR chickens, and feeding breast and thigh meat from the NC/04 SIV-inoculated WPR to WL chickens did not transmit NC/04 SIV.[169]

Changes in the amino acid composition and contents of gamma-glutamyl-beta-alanyl-histidine isopeptide in the macromolecular fraction were measured during heating of chicken extract.[170] To confirm using high-performance liquid chromatography (HPLC) analysis the formation of γ-glutamyl-β-alanyl-histidine isopeptide in the macromolecular fraction in food, the contents of the above peptide were quantified from the macromolecular fraction of heated model chicken extract and commercial chicken extract. Increases of histidine, 1-methylhistidine, and beta-alanine were observed, suggesting that carnosine and anserine were incorporated into the macromolecular fraction.[170] The increase of gamma-glutamyl-beta-alanyl-histidine isopeptide in the macromolecular fraction of chicken extract was also observed during the heating process. Furthermore, measurement of gamma-glutamyl-beta-alanylhistidine isopeptide in commercial chicken extract showed that all kinds of chicken extract contained the above isopeptide (from 0.09 to 0.31 μmol/g dry mass in the macromolecular fraction). These results suggest that the formation of gamma-glutamyl-beta-alanyl-histidine and related isopeptides occur during heating of chicken extract.

From the result obtained in this study,[170] the incorporation of carnosine into the macromolecular fraction and formation of gamma-glutamyl-beta-alanyl-histidine isopeptide also occur during heating of chicken extract. From the observations, it seemed possible that some of carnosine has been incorporated into the macromolecular fraction by a reaction between the histidine moeity and lipid-oxidation products. Furthermore, the increase in contents of 1-methylhistidine in the macromolecular fraction suggests that anserine was also incorporated into the macromolecular fraction during heating. The measurement of gamma-glutamyl-beta-alanyl-histidine isopeptide in the macromolecular fraction of various commercial meat extracts indicated that all of the commercial meat extracts tested contained the isopeptide, in concentrations ranging from 0.04 to 0.87 μmol/g of dry matter.[171] This variation was suggested to be due to the differences between the processes of extraction and the

differences in the initial amounts of carnosine. A positive correlation between the content of gamma-glutamyl-beta-alanyl-histidine and the color of the macromolecular fraction was observed. These results suggested that gamma-glutamyl-beta-alanyl-histidine is widely distributed in meat products and that the content can be used as an index of protein denaturation during the heating process.[171] To confirm the formation of gamma-glutamyl-beta-alanyl-histidine and related peptide, a model solution (amide-containing amino acids and carnosine) has been heated, and the products were investigated.[172] Spectroscopical analysis has indicated that the major product from asparagine and carnosine is beta-aspartyl-beta-alanyl-histidine, and that from glutamine and carnosine is gamma-glutamyl-beta-alanyl-histidine. Furthermore, to confirm the increase of the above peptides during the heating process of food, an HPLC method for the determination of these isopeptides in food protein was constructed. The isopeptides were liberated by proteolytic digestion and fractionated by solid-phase extraction using Toyopack IC-SP cartridges. The fraction containing the isopeptides was derivatized with phenylisothiocyanate and separated and quantified by HPLC using an octadecyl-silica column. As a result of quantification, an increase of the gamma-glutamyl-beta-alanyl-histidine isopeptide in the macromolecular fraction of heated beef soup stock solution has been observed. These results suggest that the formation of the isopeptide occurs in the heating of various foods containing carnosine. Chicken soup enriched with combination of the above cited natural peptides (carnosine, anserine, and their isopeptide derivatives) during preparation (heating of chicken extract) has long been recognized to possess unusual therapeutic potency against a wide variety of viral and bacterial agents (Figure 5).

Because it is simple to prepare, relatively cheap, nutritious, and easily digested, chicken soup is a good food for winter convalescents. Sipping warm soup can also clear the sinuses because of the steam ventilating into the nasal passages, serving as a natural decongestant, which also relieves cold and flu symptoms. Additionally, cold and flu viruses can only survive within a narrow temperature range, and sipping hot liquids can raise the ambient temperature in the nose and throat above this threshold.

It should be added that to benefit from Jewish Penicillin, one need not be Jewish. The first chicken soup was made in the poor communities in Russia where for centuries poultry was the only affordable meat. Many families used the whole bird to prepare a three-course traditional meal, starting with chopped liver, followed by a broth-like soup and finishing with the rest of the fowl for an entrée. Today, chicken soup is still served as part of a Shabbat meal in most Jewish homes, at festivals and weddings and as a general cold weather pick-me-up. Accompaniments can include cooked lokshen (vermicelli or egg noodles), kreplach (three-cornered "Jewish ravioli" filled with onion and minced beef or chicken), ravioli or knaidlech at Passover (matzoh balls) or even an egg-glazed puff pastry crust baked onto individual bowls of soup in a hot oven for 15 minutes or until the pastry is golden brown.

Indeed, as early as the 12th century, the theologian, philosopher, and physician, Moses Maimonides wrote, "Chicken soup . . . is recommended as an excellent food and medication."[173] Previous anecdotal reports regarding the therapeutic efficacy of this agent, however, have failed to provide details regarding the appropriate length of therapy. Ancient Jewish literature included descriptions of folk remedies, even the "Dreckapotheke," medicines that were derived from offensive human and animal parts and excretions (We place the swirling of dead chicken remains in vegetable broth in this category). However, in keeping with respect for educated scientific and medical opinion, Jewish sages throughout the ages have warned that one should not rely on the folk remedies of the Talmud, and some have even stated that it is forbidden to do so. Rennard et al[174] mention that chicken soup has been referred to as Jewish penicillin, and they have no doubt that it is just as effective as any antibiotic in the treatment of viral illnesses. But instead of trying to inhibit the function of white blood cells to control inflammation, which could be harmful, we should be trying to bolster the immune system to eradicate the infection and prevent complications.

Chicken soup has long been regarded as a remedy for symptomatic upper respiratory tract infections. As it is likely that the clinical similarity of the diverse infectious processes that can result in "colds" is due to a shared inflammatory response, an effect of chicken soup in mitigating inflammation could account for its attested benefits.[174] To evaluate that possibility, the ability of chicken soup to inhibit neutrophil chemotaxis in response to standard chemotactic stimuli was evaluated and demonstrated in this study. A traditional chicken soup was tested for its ability to inhibit neutrophil migration using the standard Boyden blind-well chemotaxis chamber assay with zymosan-activated serum and fMet-Leu-Phe as chemoattractants. Chicken soup significantly inhibited neutrophil migration and did so in a concentration-dependent manner. The activity was present in a nonparticulate component of the chicken soup. All of the vegetables present in the soup and the chicken individually had inhibitory activity, although only the chicken lacked cytotoxic activity.

FIGURE 5. Chicken Soup or 'Jewish Penicillin' is perhaps the ultimate comfort food. It's also one of the simplest ways to feel full and nourished. The pounding headache, the sore neck, the runny nose: all things leading to the start of a cold, and in no time flat, the cold turns into the flu. Many Americans swear on a traditional household remedy called ''Jewish penicillin.'' This remedy is no more than a simple chicken broth. The broth contains protein substances, and one in particular cysteine that helps strengthen circulation and stimulates the immune system.

Interestingly, the complete soup also lacked cytotoxic activity.[174] Commercial soups varied greatly in their inhibitory activity. The present study, therefore, suggests that chicken soup may contain a number of substances with beneficial medicinal activity. A mild anti-inflammatory effect could be one mechanism by which the soup could result in the mitigation of symptomatic upper respiratory tract infections.

These results provide one mechanistic basis in support of the traditional claims made for chicken soup as a remedy. Colds are generally the result of transient infections of the mucosa of the upper respiratory tract with a variety of viruses including, but not limited to, the rhinoviruses.[175–177] Although incompletely understood, the viral infection leads to the stimulation of a cytokine cascade.[178,179] It is likely that many, if not most, of the symptoms related to colds are consequent to the inflammatory response thus initiated.[180–182]

The activation of common physiologic pathways likely accounts for the marked similarity of symptoms that result from colds. In this regard, colds are associated with the generation of neutrophil chemotactic activities[178–180,182] and with the recruitment of neutrophils to the epithelial surface of the airways.[179,180,183,184]

Because neutrophil products are potent secretagogues,[185] this may be one mechanism by which colds commonly lead to cough and sputum from a diverse set of infections. Chicken soup may have a number of beneficial effects for an individual with a cold. These could include actions as diverse as improving hydration and nutritional status[186] and accelerating mucosal clearance.[187] The warm liquid, particularly when sipped, can stimulate nasal clearance and may improve upper respiratory tract symptoms. However, the preparation of the soup is a multistep process, and many complex chemical interactions are taking place. Determining these processes quantitatively and preparing appropriately controlled component extracts will be a challenging problem.

A traditional ''canonic'' chicken soup is prepared according to a family recipe, which will be referred to as ''Grandma's soup'' (C. Fleischer; personal communication; 1970). This recipe is as follows:

1 5- to 6-lb stewing hen or baking chicken;
1 package of chicken wings;
3 large onions;
1 large sweet potato;
3 parsnips;
2 turnips;
11 to 12 large carrots;
5 to 6 celery stems;
1 bunch of parsley; and
Salt and pepper to taste.

Clean the chicken, put it in a large pot, and cover it with cold water. Bring the water to a boil. Add the chicken wings, onions, sweet potato, parsnips, turnips, and carrots. Boil about 1.5 hours. Remove fat from the surface as it accumulates. Add the parsley and celery. Cook the mixture about 45 minutes longer. Remove the chicken. The chicken is not used further for the soup. (The meat makes excellent chicken parmesan.) Put the vegetables in a food processor until they are chopped fine or pass through a strainer.

Salt and pepper to taste. (Note: this soup freezes well.) Matzoh balls were prepared according to the recipe on the back of the box of matzoh meal (Manischewitz; Jersey City, NJ).

The therapeutic efficacy of chicken soup was first discovered several thousand years ago when an epidemic highly fatal to young Egyptian men seemed not to affect an ethnic minority residing in the same area. Contemporary epidemiologic inquiry revealed that the diet of the group not afflicted by the epidemic contained large amounts of a preparation made by boiling chicken with various vegetables and herbs. It is notable in this regard that the dietary injunctions given to Moses on Mount Sinai, while restricting consumption of no less than 19 types of fowl exempted chicken from prohibition.[188] Some scholars believe that the recipe for chicken soup[189] was transmitted to Moses on the same occasion, but was relegated to the oral tradition when the Scriptures were canonized. Chicken soup was widely used in Europe for many centuries, but disappeared from commercial production after the Inquisition. It remained as a popular therapy among certain Eastern European groups, however, and was introduced into the United States in the early part of 20th century. Although chicken soup is now widely employed against a variety of organic and functional disorders, its manufacture remains largely in the hands of private individuals, and standardization has proved nearly impossible.[189,190]

Preliminary investigation into the pharmacology of chicken soup (Bohbymycetin®) has shown that it is readily absorbed after oral administration, achieving peak serum levels in 2 hours and persisting in detectable levels for up to 24 hours. Parenteral administration is not recommended. The metabolic fate of the agent is not well understood, although varying proportions are excreted by the kidneys, and dosage should be appropriately adjusted in patients with renal failure. Chicken soup is distributed widely throughout body tissues and breakdown products having antimicrobial efficacy cross the blood–brain barrier. Untoward side effects are minimal, consisting primarily of mild euphoria which rapidly remits on discontinuation of the agent. Although chicken soup has been employed for thousands of years in the treatment of viral and bacterial illnesses, there have been no systematic investigations into the optimal course of therapy.[189,190] Evidence-based medicine applies the best evidence from controlled trials, uncontrolled studies and case reports and purports to consider the patient's values and preferences regarding treatment options as well. We think the popularity of chicken soup, as evidenced by observational data and the experience of generations of patients and healers, shows overwhelmingly that patients value and prefer this remedy for a number of conditions and ailments. Among the adverse effects of chicken soup we found reports of hypernatremia,[191,192] anaphylaxis[193] various minor effects noted in the writings of Maimonides and listed by Rosner[194] and the unusual case of a chicken bone being lodged in the bronchus of a child who had choked while drinking unstrained chicken soup for his pneumonia.[195]

All in all, the anecdotal evidence advocating the benefits of chicken soup far outweighs that describing its shortcomings.

Table 1. Ingredients of a standard chicken soup for home medicine.

3 chicken carcasses and 500 g of giblets including chicken feet
1 large onion, quartered
2 carrots, chopped
1 leek, chopped
2 celery stalks, chopped
Parsley—stalks for soup, flowers for garnish
4 peppercorns or to taste
2 bay leaves
150 g of vermicelli rice noodles
Method
Put all ingredients except the vermicelli in a large saucepan and cover with water. Bring to the boil, then skim to remove any scum from the surface. Simmer for two and a half hours and remove scum once or twice during that time.
When cooked, strain the broth and remove the fat floating on top with kitchen paper. Add vermicelli and simmer. The noodles should only take a minute or so to soften. Throw on some parsley, grab a good book or magazine and savor the simple life.

(WO 2004/028536 A1; WO 94/19325; WO 95/12581; WO 2004/064866 A1)

Can-C Plus 90 caps	Item Code: 0629

Supplement facts:
Serving Size: 3 capsules

Amount Per Serving	%	DV
N-acetylcysteine	600mg	*
L-histidine	300mg	*
Carnisine	210mg	*
Vitamin E	150IU	498%
D-pantethine	90mg	894%
L-methione	75mg	*
Zinc picolinate	15mg	99%

% Daily Value * not established
Other ingredients: Rice flour, magnesium sterate, vegetable capsule.

Note: Keep in cool dark conditions, out of the reach of children and consume before end of expiry date. Not for use by pregnant or lactating women.

FIGURE 6. Nonhydrolized in digestive tracts and blood natural carnosine peptide as a panacea of tomorrow for various flu ailments. Nonhydrolized carnosine (carnisine) exerts the signaling activity attenuating nitric oxide production, cytostasis and NO-dependent inhibition of Influenza virus replication in macrophages and inhibits inflammatory response and tissue damage in the respiratory tract due to the intrinsic fundamental properties of L-carnosine including direct NO-trapping ability and directly by modulating cytokine release. This formulation of ingredients represents stable mimics of the biologically active carnosine suitable for pharmacological applications.

CONCLUSIONS

Respiratory virus infection causes airway inflammation and bronchial asthma exacerbation.[196,197] The pathogenesis of airway inflammation caused by respiratory viruses is complex and involves multiple inflammatory cells, cytokines, and mediators.[196,197] The earliest host responses to viral infections are nonspecific and involve the induction of cytokines, among them, IFNs, and TNF-α. Gamma IFN (IFN-g) and TNF-α have both been shown to be active in many cell types and induce cascades of downstream mediators. Airway epithelial cells are the initial site of respiratory virus infection and have the capacity to produce a variety of biologically active molecules including cytokines.[198] Influenza virus which is one of the major respiratory viruses, also causes airway inflammation and exacerbates asthma.[196–200] Epithelial cells can function maintain mucosal integrity and to modulate local immune responses. They can also limit inflammatory processes by degrading, or inhibiting, proinflammatory mediators and proteins. However, the epithelium also responds to a range of stimuli by producing biologically active mediators that can influence airway inflammation. These include, but are not limited to a broad range of cytokines and chemokines that can exert profound effects on inflammatory cells, and lipid and peptide mediators.

This review has not been devoted to the different strategies viruses have taken to promote their transmission or survival but rather to one aspect of the innate immune response to infection: the role of NO in the influenza antiviral repertoire. Recently, data from many laboratories, using both RNA and DNA viruses in experimental systems, have implicated a role for NO in the immune response. The data do not indicate a magic bullet for all systems but suggest that NO may inhibit an early stage in viral replication and thus prevent viral spread, promoting viral clearance and recovery of the host. In this review the role of NO in the pathogenesis of influenza virus–induced respiratory diseases was investigated. The results strongly suggest formation of peroxynitrite in the lung through the reaction of NO with $O_2^{-\cdot}$, which is generated by alveolar phagocytic cells and xanthine oxidase. It has been shown that free radicals, such as the superoxide anion and NO, are pathogenic molecules in viral disease.

Much attention has been given to a critical role of nonhydrolized forms of L-carnosine acting therapeutically (Figure 6)[201] through NO and cytokines regulating activities of this vitally important dipeptide in the pathologic events of influenza-induced various inflammatory respiratory diseases. In the present study, we evaluated the potent effects of nonhydrolized carnosine on influenza-virus–induced pneumona infected with a dose of a swine influenza virus A (H1N1). The administration of nonhydrolized carnosine in the patented oral health care formulations,[201] significantly improve the therapeutic value of influenza virus–induced

pneumonia and survival rate, by inhibiting inflammatory cell responses and suppressing NO overproduction in the lung.

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