

Ouachita Baptist University

Scholarly Commons @ Ouachita

Honors Theses

Carl Goodson Honors Program

2008

Resveratrol Modulation of Microglial Activation and Provision for Neuroprotection in Multiple Sclerosis

Joseph L. Green

Ouachita Baptist University

Follow this and additional works at: https://scholarlycommons.obu.edu/honors_theses



Part of the [Diseases Commons](#)

Recommended Citation

Green, Joseph L., "Resveratrol Modulation of Microglial Activation and Provision for Neuroprotection in Multiple Sclerosis" (2008). *Honors Theses*. 62.

https://scholarlycommons.obu.edu/honors_theses/62

This Thesis is brought to you for free and open access by the Carl Goodson Honors Program at Scholarly Commons @ Ouachita. It has been accepted for inclusion in Honors Theses by an authorized administrator of Scholarly Commons @ Ouachita. For more information, please contact mortensona@obu.edu.

SENIOR THESIS APPROVAL

This Honors thesis entitled

“Resveratrol Modulation of Microglial Activation and Provision for Neuroprotection in Multiple Sclerosis”

written by

Joseph L. Green

and submitted in partial fulfillment of the requirements for completion of the Carl Goodson Honors Program meets the criteria for acceptance and has been approved by the undersigned readers.

(Name) thesis director

(Name) second reader

(Name) third reader

honors program director

Resveratrol Modulation of Microglial Activation and Provision for Neuroprotection in Multiple Sclerosis

Joseph L. Green

Abstract

Multiple sclerosis (MS) is an autoimmune disease affecting over 2.5 million people worldwide. In MS, myelin sheaths surrounding the axons of neurons are attacked by the immune system, leading to many deleterious symptoms. No cure for MS has been found, but some treatments reducing the number of exacerbations are available. Studies on resveratrol show the compound to have anti-inflammatory properties that could be beneficial in the treatment of diseases like MS. In our research, we used a cellular model to imitate neurons of the central nervous system and tested the effects of resveratrol in the hope of finding neuroprotective properties.

Introduction

MS

Multiple Sclerosis affects approximately 350,000 people in the United States, 2.5 million people worldwide, and is the most prevalent neurological cause of disability in young adults (Pinkston, Kablinger et al. 2007). MS occurs more frequently in females than in males and is more prevalent in some countries than in others (Poser 1994). Even though research has led to advancements that are minimizing the many mysteries of this disease, its cause is unknown and a cure is still not available.

MS is an autoimmune disease in which an inflammatory response of unknown origin causes the immune system to begin a series of attacks on the central nervous system, leading to loss of oligodendrocytes, cells that function to create and maintain the

myelin sheath surrounding the axons of nerve cells (McQualter and Bernard 2007). The loss of these cells results in demyelination and axonal degeneration and the formation of demyelinated lesions because there are an insufficient number of the cells to completely remyelinate the axons of the nerve cells (Wolswijk 2000). These demyelinated lesions decrease the efficacy of the electrical signal that is passed from the nerve cells, a decrease that is thought to elicit the symptoms normally seen in MS: paralysis, sensory loss, fatigue, depression, movement problems, tremors, weakness in arms or legs, loss of balance, and many cognitive defects (McQualter and Bernard 2007).

The lesions in the white matter of the brain characteristic of MS are a result of a defective blood brain barrier, which, when functioning properly, controls entry of certain molecules into the brain (Dutta and Trapp 2007). In MS, the blood brain barrier allows certain MHC class-II positive cells to pass through its tight junctions, resulting in the arrival of a type of white blood cell called T cells that attack the brain parenchyma, causing the neurological damage associated with MS (Sospedra and Martin 2005). The damage done to the axons characteristic to MS is believed to occur secondarily to the inflammation response. $CD4^+$ T cells, $CD8^+$ T cells, macrophages, and glial cells are normally responsible for the secretion of cytokines, chemokines, and other proteins that mediate the inflammatory response (Taoufik, Tseveleki et al. 2008).

The major histocompatibility complex (MHC) is a group of genes that code for human lymphocyte antigens (HLA) that line the surface of all nucleated cells. The HLA proteins are responsible for the immune system's recognition of "self" and non-self." Therefore, a defect in the HLA system can result in an autoimmune disease. B cells and macrophages are examples of the antigen-presenting cells belonging to the MHC class-II

positive cells. These MHC class-II complexes are recognized by specific mature helper T cells that express the surface protein CD4, called CD4⁺ T cells. CD4⁺ T cells release cytokines that signal other immune cells, therefore amplifying the immune response (Cresswell 1998).

The inflammatory response in MS is triggered when the MHC Class-II positive cells that have passed through the blood brain barrier come in contact with an antigen present on microglia or astrocytes. This contact initiates the release of many types of proinflammatory molecules such as chemokines and cytokines that decrease the permeability of the blood brain barrier, allowing immune cells to enter. The regulatory CD4⁺ T cells, along with triggering the inflammatory response, may also aid in the reduction of oligodendrocytes and axonal degeneration (McQualter and Bernard 2007).

The different lesions frequently observed in MS can be divided into three types. One type, active lesions, contain a high number of MHC Class-II positive cells and are generally newly formed lesions approximately a few months old. The cells found in these lesions are macrophages filled with lipids, mostly cholesterol. The difference between this type of lesion and the second type, chronic active lesions, is the myelin remains left by the macrophages located at the edge of the chronic active lesions. The third type, chronic inactive lesions, is distinguished by its low number of MHC Class-II positive cells in comparison to the two other types of lesions. The lesions with the higher numbers of MHC Class-II positive cells amplify the inflammatory response, leading to an increased amount of tissue damage (Dutta and Trapp 2007).

MS is characterized by impairment of autonomic, motor, and neurocognitive function, but the severity of the impairments varies in individuals suffering from the

disease ((Sospedra and Martin 2005). Losses of cognitive function occur in about 70 percent of patients with examples such as long-term memory loss, inability to maintain attention, and slower time in processing information (Simioni, Ruffieux et al. 2007). Though MS does not necessarily shorten the life of an individual suffering from it, the possible deleterious symptoms can arise without warning and make normal life difficult. Individuals with multiple sclerosis experience different symptoms depending on the nerves affected by active areas of inflammation (Sospedra and Martin 2005).

Giving a prognosis to an individual with MS is very difficult because MS can remain dormant for a long period of time or progress at a fast pace. The different types of MS are categorized depending on number, severity, and occurrence of attacks on the central nervous system. After a single attack, an individual would be diagnosed with a clinically isolated syndrome. Approximately 85 percent of people with MS suffer from the relapsing-remitting form in which the central nervous system is attacked without warning and symptoms are present, but is followed by a period free of attacks. The damage inflicted as a result of these attacks could lead to permanent symptoms, but other symptoms are relieved after the attacks cease. Most people suffering from the relapsing-remitting type of MS develop the secondary progressive form of MS. In secondary progressive MS, the patient experiences a worsening of symptoms with no clear absence of the attacks associated with the disease. Some individuals with this type do experience a period of remission. Patients that never experience a remission after their first sign of symptoms and show a progressive worsening of symptoms suffer from primary progressive MS, which is common in people diagnosed with MS later in life. Those individuals with progressive MS that suffer another relapse are then categorized as

having progressive-relapsing MS, which is diagnosed less frequently than the other types. The factors that trigger the relapses are still unknown, but studies have shown several factors such as the common cold, influenza, and emotional or physical stress can possibly contribute to the unpredictable attacks (Murray 2006).

Etiology of MS

Despite the amount of research on the disease, the exact cause of the inflammatory response and autoimmune attack in MS is unknown. Several theories have been proposed, but none has presented enough evidence to solve this mystery.

Genetic factors have been theorized to be a cause of MS, but the research has been unsuccessful in finding definitive parallels in the genomes of individuals with the disease. There have been substantial findings that suggest genetic does play a role in susceptibility to MS. One such finding shows increased susceptibility to the disease for persons of North European descent. This study compared ethnic groups living in the same area. Other findings show an increased risk in close relatives of individuals suffering from MS; they are up to 40 times more likely to have the disease. Also, an identical twin of a person with multiple sclerosis is approximately 30 percent more inclined to get the disease than a fraternal twin, which only has around a seven percent chance. Researchers believe the mode of inheritance is likely through genes on multiple loci each contributing to the risks associated with multiple sclerosis (Haines, Terwedow et al. 1998).

Not all gene-association studies are in agreement about which genes play a vital role in multiple sclerosis. The genetic region coding for the HLA system has been found by many genetic linkage studies to be one of the contributors to the susceptibility of MS.

In a recent study, a connection was found regarding the genes responsible for the production of the major histocompatibility complex cells that permeate the blood brain barrier and are believed to be a major cause of the neurological damage seen in MS (Hafler, Compston et al. 2007). The HLA DR and HLA DQ class II genes, specifically the class II DRB1*1501-DQA1*0102-DQB1*0602 haplotype on chromosome 6p21, are thought to give individuals a predisposition to the disease (Barcellos, Oksenberg et al. 2002). Recent studies have shown the locus association is more in the HLA DR Class II region than in the HLA DQ region. The class II region is responsible for encoding for the antigen-presenting proteins located on the outside of the cell for recognition by T cells. Other gene-association studies have shown linkage on chromosomes 5q33, 17q23, and 19p13. Polymorphisms of the cytokine interferon-gamma and apolipoprotein E are thought to be a part of the autosomal yet gender-specific cause of multiple sclerosis (Kantarci and Wingerchuk 2006).

Environmental factors are also thought to play a role in the etiology of multiple sclerosis. Geographical association for the disease is one type of evidence supporting this cause of MS. Multiple sclerosis is rare in Asia and is more common as latitude increases. Also, MS is hardly found in tropical regions. An infectious agent, such as a type of bacteria or virus, has long been hypothesized to be a main cause of multiple sclerosis. There are three hypotheses postulating how a common infectious agent causes multiple sclerosis. The first, the *poliomyelitis hypothesis*, states that an infant who catches the virus early in infancy acquires protective immunity. However, if the virus is caught in late adolescence or in adulthood it can be more harmful. The *prevalence hypothesis* states that a virus found in areas where multiple sclerosis is prevalent causes the disease.

The *poliomyelitic hypothesis* has developed recently into the “hygiene” hypothesis, which postulates that exposure to many infectious agents can increase the likelihood of an individual getting MS. Some infectious agents thought to increase the risk of MS are the Epstein-Barr Virus, *Chlamydia pneumoniae*, and the Human Herpes Virus 6. There have been more consistent findings with studies done on the Epstein-Barr Virus. Persons with a history of infectious mononucleosis, which is caused by the Epstein-Barr Virus, are more likely to have MS than individuals without a history of the virus. Recent studies on *Chlamydia pneumoniae* show that exposure to this infectious agent might not increase the risk of the disease because its DNA was not found in the cerebrospinal fluid in victims of multiple sclerosis. The Human Herpes Virus 6 is still thought to increase the risk of the disease because it was found in MS lesions in the brain in MS patients after death (Ascherio and Munger 2007).

Other environmental influences that are thought to increase an individual’s chances of having multiple sclerosis are lower levels of sunlight exposure and insufficient vitamin D intake. Studies have shown that individuals who live in areas with higher sunlight or have outdoor occupations are less likely to be diagnosed with multiple sclerosis (Freedman, Dosemeci et al. 2000). Decreased risk of having MS due to increased sunlight is expected to correlate with the finding that vitamin D deficiency increases the risk of multiple sclerosis. The UVB radiation from sunlight exposure leads to the photosynthesis of vitamin D from its pre-active form previtamin D₃ to its active form vitamin D₃. Vitamin D is thought to have these effects on autoimmune disorders because high levels of vitamin D are needed for the maximum secretion of the hormone 1,25(OH)₂D, which is a very effective modulator of the immune system (Holick 2004).

Another risk factor for multiple sclerosis is cigarette smoking. Experiments have shown that heavy smokers are more likely to get MS than people who have never smoked, and smoking may cause a worsening of symptoms in MS patients. Also, smoking has been shown to speed the changeover from the relapsing-remitting to secondary-progressive MS (Ascherio and Munger 2007).

Research on the effects of sex hormones such as estrogen and progesterone on multiple sclerosis was increased when studies showed that the MS attacks decreased during pregnancy, most significantly during the third trimester when estrogen and progesterone are at their maximums. Estrogen has been shown to increase immune system responses at low concentrations, but decrease immune system responses at high concentrations. Sex hormones have the ability to affect the immune system by activating certain lymphocytes and controlling the type of antigens presented on certain cells. Based on these discoveries, sex hormones are thought to play a role in the susceptibility of people for multiple sclerosis (Whitacre 2001).

Treatments for MS

Although no cure for MS has been found, many treatment options have shown positive results for slowing the progression of the disease and providing neuroprotection during the attacks associated with multiple sclerosis. There are different treatment options for people with the relapsing form and the progressive form of the disease. (Staff 2006).

Beta interferons are currently being used as a treatment option for patients with relapsing-remitting MS. Interferon-beta 1- β and interferon-beta 1- α are man-made proteins identical to naturally occurring proteins in the body that function in modulating

the immune system and aid in fighting viral infections. The interferon beta 1- β drug available is called Betaseron. The interferon 1- α drugs available are called Avonex and Rebif. Betaseron and Rebif are taken subcutaneously, whereas Avonex is taken intramuscularly. The beta interferon drugs have been shown to decrease the immune activity of MS, resulting in a reduction in the number of relapses (Flechter, Vardi et al. 2002). Beta interferon drugs are prescribed for ambulatory MS patients who experience multiple flare-ups in a years' time. These drugs do not repair any damage that occurred prior to taking the drug. Many patients have problems with the flu-like side effects normally felt while taking the drugs. In some patients, beta interferon drugs cannot be administered due to their decreased efficacy or if the drug is harmful to their immune system. Also, some patients develop antibodies to the beta interferon drugs and are unable to continue taking them. Therefore, drugs such as glatiramer acetate provide another treatment option in MS patients (Staff 2006).

Glatiramer acetate (Copaxone) is also a synthetic protein modeled after the amino acid motifs of the myelin basic protein. Glatiramer acetate is helpful to multiple sclerosis patients by providing the same benefits as the beta interferon drugs, namely retarding the progression of the disease and the number of flare-ups as well as decreasing the activity in the lesions associated with the disease (Debouverie, Moreau et al. 2007). Glatiramer acetate works by drawing helper T cells to sites of inflammation, where they are activated and release various cytokines such as interleukin-(IL)-4, IL-5, and IL-13 that act to protect the central nervous system. These cells have also been found to release a brain-derived neurotrophic factor that may help repair neurons previously damaged by multiple

sclerosis. Copaxone is injected subcutaneously once every day (Neuhaus, Farina et al. 2001).

Interferon beta and glatiramer acetate have been approved and are the first-line agents prescribed by physicians. However, if these two drugs cannot be tolerated or are not helpful to patients, Natalizumab may be prescribed to patients with aggressive, relapsing MS. Natalizumab is injected intravenously once a day and has been shown to decrease the number of exacerbations in MS patients by reducing the inflammatory response in the central nervous system, thus not allowing the immune cells to cross the blood brain barrier. However, this drug is normally prescribed only to MS patients for whom no other treatment option has been successful due to the finding during a clinical trial that some patients developed a potentially fatal disease called progressive multifocal leukoencephalopathy (Rudick, Stuart et al. 2006).

Mitoxantrone, a drug normally prescribed for cancer patients, is an immunosuppressive drug targeting immune cells such as T and B cells and macrophages. Mitoxantrone has been shown to slow down the progression of MS, specifically the demyelination associated with the disease. Mitoxantrone is prescribed for MS patients with relapsing-remitting, secondary progressive MS, or progressive-relapsing MS and is taken intravenously every three months. Many doctors refrain from prescribing this drug due to its possibly serious adverse side effects, such as leukemias or cardiotoxicity, if taken too long. Therefore, mitoxantrone is taken for a maximum of three years to decrease the risk of a patient experiencing these side effects. The MS patient taking the drug must be monitored closely to see if the correct dosage is being taken and to assess the risks and benefits of the drug (Rizvi, Zwibel et al. 2004).

A doctor may prescribe other medications to help relieve some of the symptoms of multiple sclerosis. To help with muscle pains from tonic spasms due to MS, a doctor may prescribe antispastic agents such as baclofen or tizanidine (Pollmann and Feneberg 2008). For improvements in fatigue related to multiple sclerosis, amantidine hydrochloride has been shown to help increase energy, concentration, and problem-solving (Cohen and Fisher 1989). Some MS patients take physical and occupational therapy to help strengthen their bodies and become adjusted to living with the disease.

Resveratrol

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a polyphenolic compound that may be the new “wonder drug” due to its many potential beneficial properties concerning the treatment of diseases. Resveratrol, found in mulberries, peanuts, and red grapes, is used by plants as an antibiotic compound to fight fungal infection. Recently resveratrol has been found to have anticancer, antioxidant, and anti-inflammatory properties. Therefore, resveratrol could be used as a treatment option for multiple sclerosis due to its ability to decrease inflammation. Experimental autoimmune encephalitis, or EAE, is an inflammatory disease commonly used in studies of multiple sclerosis due to its many similarities to the disease (Imam and Kuntzel 1977). Studies of resveratrol on EAE-induced mice showed that the compound lowered the secretion of several cytokines and chemokines such as interleukin (IL)-2, IL-9, IL-12, IL-17, tumor necrosis factor- α , interferon- γ , macrophage inflammatory protein-1 α (MIP-1 α), and monocyte chemoattractant protein-1 (MCP-1). Many of these cytokines and chemokines are believed to be pro-inflammatory mediators (Singh, Hegde et al. 2007). One of the possible ways resveratrol works is by suppressing the inflammatory response through the

release of pro-inflammatory mediators and its ability to inhibit the synthesis of these mediators through its effects on nuclear factor kB (NF-kB) or activator protein (AP-1). Nuclear factor kB is responsible for the increased expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Nitric oxide and cyclooxygenase are enzyme systems responsible for the synthesis of many pro-inflammatory mediators in the body. Nuclear factor kB is a transcription factor for genes involved in inflammation, whereas AP-1 is a transcription factor for genes involved in cell differentiation (de la Lastra and Villegas 2005).

Neuroprotection

Beta-interferon and glatiramer acetate treatments are available for slowing the progression of multiple sclerosis, but there are not any drugs available that have shown conclusive ability to provide neuron protection. A drug with neuroprotective properties could protect the neurons from damage during an exacerbation of MS resulting in the disappearance of the symptoms associated with the disease. Although more studies on the neuroprotective effects of resveratrol should be performed, resveratrol seems to protect neurons from damage in cerebral ischemia by raising the nitric oxide level and lowering the hydroxyl radical level. Though this finding may seem contradictory due to information previously stated about raising the nitric oxide level leading to harmful effects in MS, the higher nitric oxide level is helpful because nitric oxide is a vasodilator. Therefore, nitric oxide causes an increase in blood flow that is crucial in the event of a stroke (Lu, Chiou et al. 2006)

Neuroblastomas as Model for Neurons in Multiple Sclerosis

Fully developed neurons are no longer capable of dividing. Therefore, using neurons in the studies of multiple sclerosis is impractical and presents many problems for researchers studying autoimmune diseases such as multiple sclerosis. Neuroblastoma cells are sometimes used in studies of MS because these cancer cells are constantly dividing and exhibit similar properties to the fully developed neurons of the central nervous system that are damaged in the attacks of multiple sclerosis. Neuroblastoma is a childhood cancer of the sympathetic nervous system and is ideal for cell models for its ability to go through chemically induced cell differentiation (Abemayor and Sidell 1989).

Methods

Cell Culture

HAPi microglia cells were cultured at 37°C and 5.0% CO₂ in Dulbecco's Modification of Eagle's Medium with Earle's salts containing a final concentration of 10% fetal bovine serum and 1.38 mM L-glutamine.

Primary Cell Culture

Primary mouse microglia cultures were obtained through a modification of the McCarthy and de Vellis protocol. Cerebral cortices from 1- to 3-day-old C57BL/6 mice were excised, meninges removed, and cortices minced into small pieces. Cells were separated by trypsinization followed by trituration of cortical tissue. The cell suspension was filtered through a 70-µm cell strainer to remove debris. Cells were centrifuged at 153×g for 5 min at 4°C; resuspended in DMEM medium containing 10% FBS, 1.4 mM l-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, OPI medium supplement, and 0.5 ng/mL recombinant mouse GM-CSF; and plated into tissue-culture flasks. Cells were

allowed to grow to confluence (7–10 days) at 37°C/5% CO₂. Flasks were then shaken overnight (200 rpm at 37°C) in a temperature-controlled shaker to loosen microglia and oligodendrocytes from the more adherent astrocytes. These less-adherent cells were plated for 2–3 hr and then lightly shaken to separate oligodendrocytes from the more-adherent microglia. Microglia were seeded in 96-well plates (4×10^4 cells/well) and incubated overnight at 37°C/5% CO₂. Astrocytes were recovered by trypsinization and seeded into 96-well plates (1×10^5 cells/well).

Determination of Cell Viability

An MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) assay measuring mitochondrial activity was used to assess cell viability. MTT solution was prepared by diluting MTT 1:50 with culture medium. Cell culture media were removed from cells and replaced with 500 μ L MTT solution and incubated at 37 °C for 1 hr. MTT solution was removed and cells were lysed by adding 500 μ L DMSO/well. Plates were rocked for 30 min and read on a plate reader at 570 nm.

Determination of Nitrite Production

Cells were plated in a 24-well plate at 2.5×10^5 cells/well and incubated for 24 hr before treatment with RES. Cells were then incubated 4 hr before inducing activation of HAPi microglial cells with LPS. Media was harvested from wells after 24 hr; 50 μ L of each sample were added to a 96-well plate with 50 μ L Gries reagent and read on a plate reader at 550 nm.

IL-6 Elisa Assay

IL-6 production by microglia was determined according to manufacturer's recommendations (BD Biosciences-PharMingen, San Diego, CA). For analysis of IL-6,

microwells were coated with 100 μ L per well of Capture Antibody diluted in Coating Buffer. The plate was sealed and incubated overnight at 4 $^{\circ}$ C. The wells were aspirated and washed three times with 300 μ L/well of wash buffer. The plates were then blocked with 200 μ L/well of Assay Diluent. The plate was then incubated at room temperature for 1 hr. The wells were again aspirated and washed three times with 300 μ L/well of wash buffer. Standard and sample dilutions in Assay Diluent were prepared. 100 μ L of each standard, sample, and control were pipetted into appropriate wells. The plate was sealed and incubated for 2 hrs at room temperature. The wells were again aspirated and washed, but with a total of five washes. 100 μ L of Working Detector (Detection Antibody + SA_v-HRP reagent) were added to each well, and the plate was sealed and incubated for 1 hr at room temperature. The wells were then washed again, but with seven total washes. 100 μ L of Substrate Solution were then added to each well. Then, the plate was left to incubate unsealed in the dark for 30 min. at room temperature. 50 μ L of Stop Solution were added to each well. The absorbance was read at 450nm within 30 min. of stopping the reaction.

Results

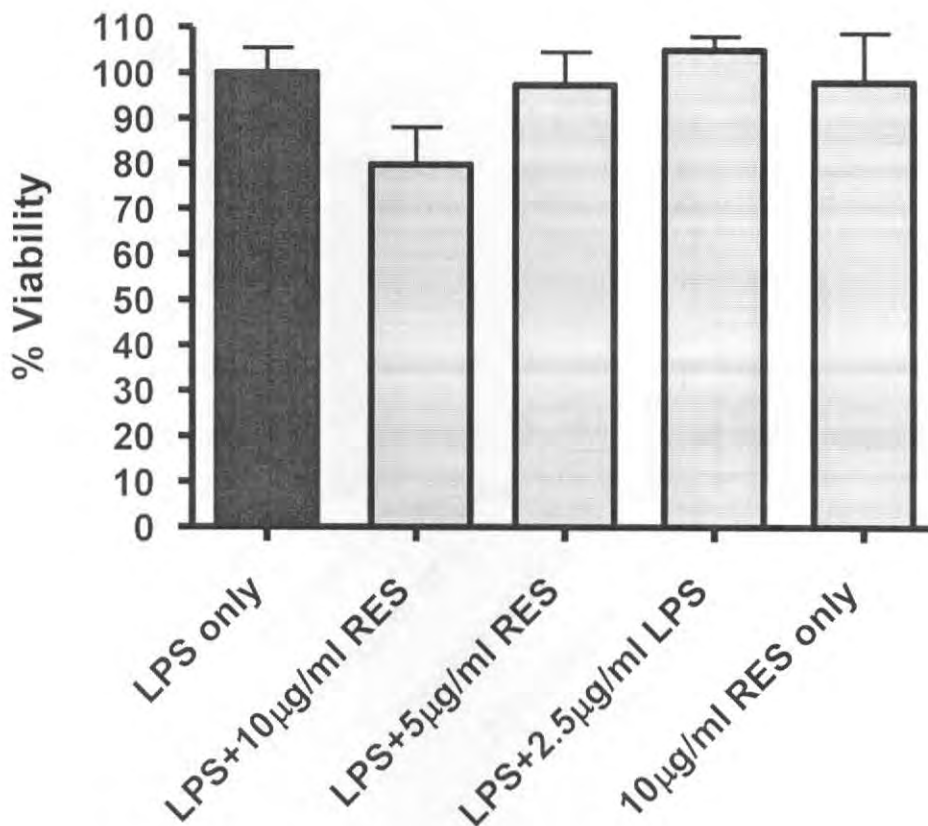


FIG 1. Resveratrol does not affect the viability of HAPi microglial cells induced by LPS.

Cells were pre-treated for 1 hr with the indicated concentrations of RES. LPS was added at 0.5 mg/mL and 24 hr later cell viability was determined. Values represent the average for triplicate cultures. Standard errors are indicated. No statistically significant differences in viability between samples were observed.

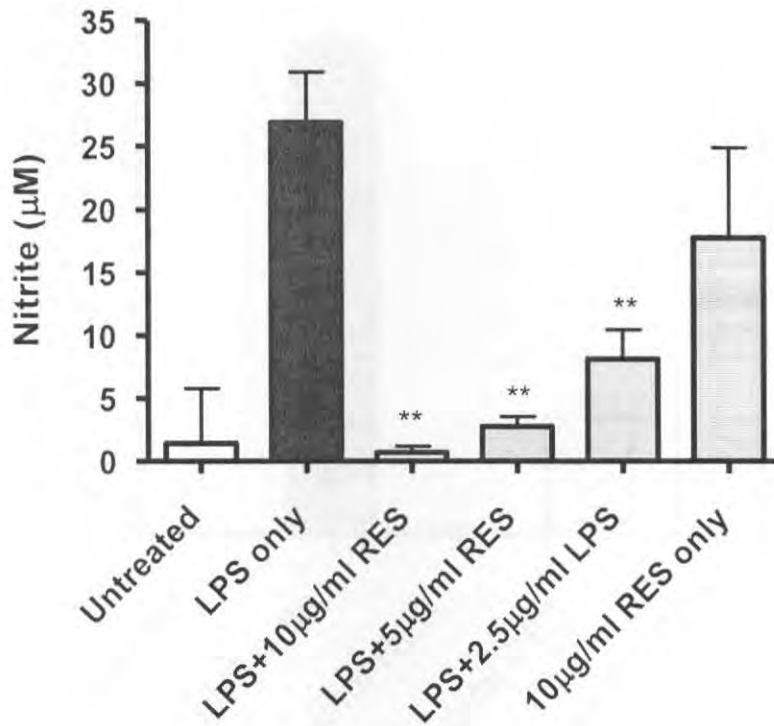


FIG 2. Resveratrol inhibits LPS induction of nitrite in HAPI microglia cells.

Cells were pre-treated for 1 hr with the indicated concentrations of RES. LPS was added at 0.5 mg/mL and 24 hr later the concentration of nitrite in the culture media was determined. Values represent the average for triplicate cultures. Standard errors are indicated. Comparisons were made to LPS-only treated cultures. Asterisks indicate statistical significance of $p < .05$.

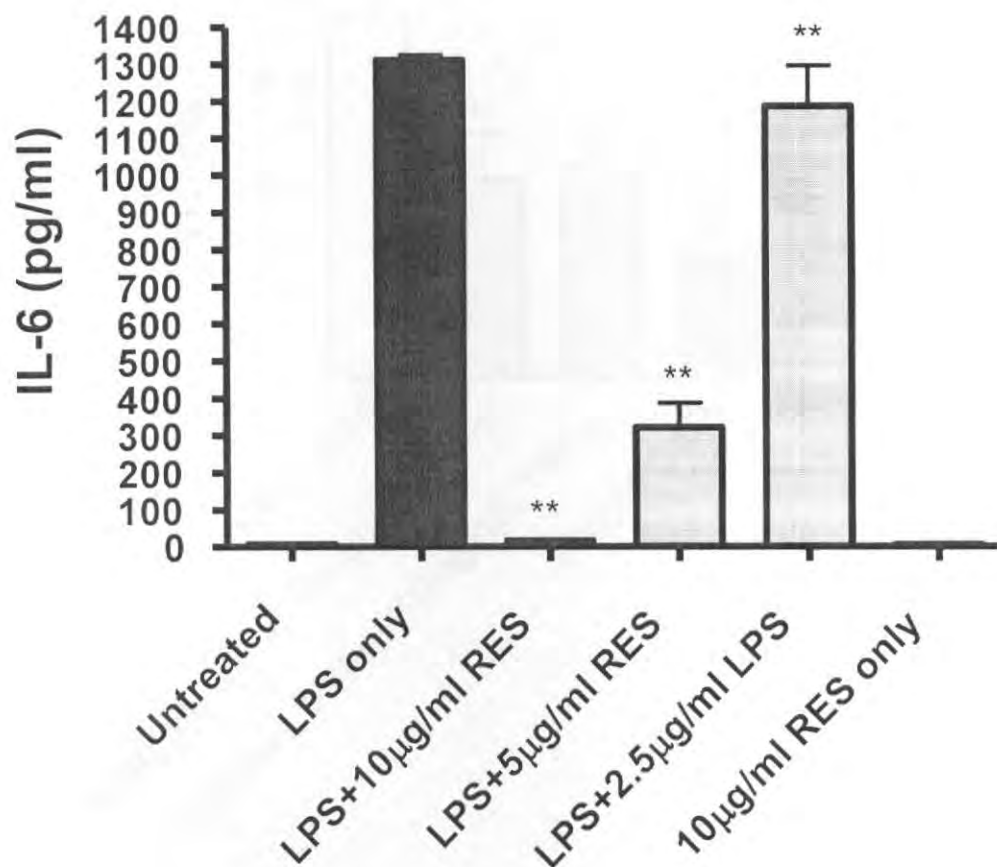


FIG 3. Resveratrol inhibits LPS induction of IL-6 in HAPI microglia cells. Cells were pre-treated for 1 hr with the indicated concentrations of RES. LPS was added at 0.5 mg/mL and 24 hr later the concentration of IL-6 in the culture media was determined. Values represent the average for triplicate cultures. Standard errors are indicated. Comparisons were made to LPS-only treated cultures. Asterisks indicate statistical significance of $p < .05$.

Similar data were observed for IL-12p40, TNF- α , MCP-1, and IL-1B (in primary microglia).

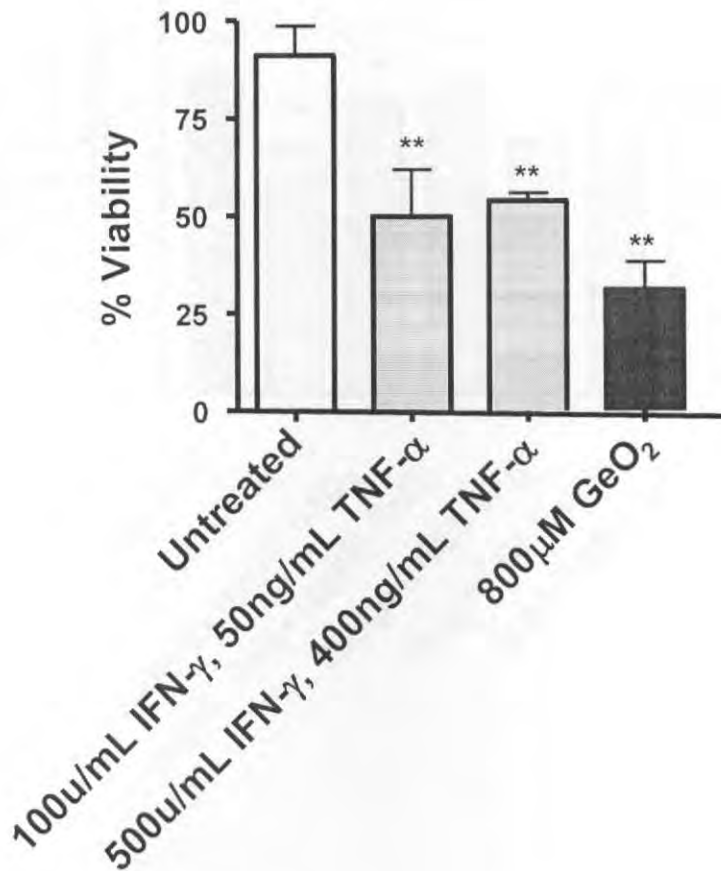


FIG 4. IFN- γ /TNF- α cocktails and GeO₂ can induce apoptosis in neuro-2a

neuroblastoma cells. IFN- γ /TNF- α or GeO₂ was added to culture medium at indicated concentrations and 72 hr later cell viability was determined. Values represent the average for triplicate cultures. Standard errors are indicated. Asterisks indicate statistical significance of $p < .05$ as compared to untreated samples. Although these apoptosis agents initially showed promise in our system, they did not consistently and reproducibly induce death in our neuro-2a cells.

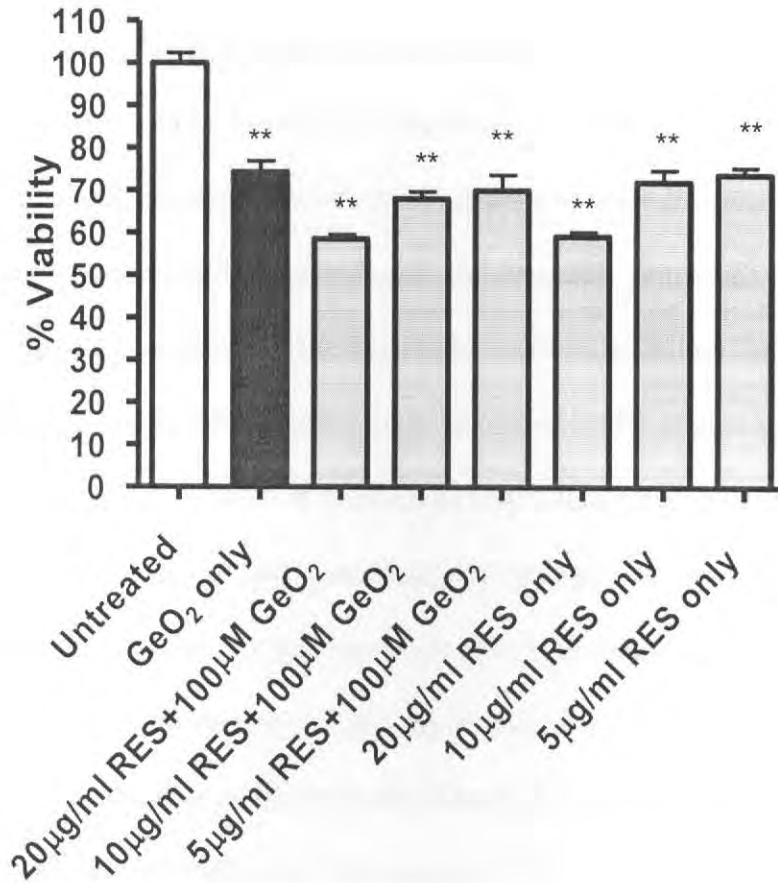


FIG 5. Resveratrol can induce apoptosis in neuro-2a neuroblastoma cells. Cells were pre-treated with the indicated concentrations of resveratrol for 1 hr. GeO₂ was added to culture medium at indicated concentrations and 72 hr later cell viability was determined. Values represent the average for triplicate cultures. Standard errors are indicated. Asterisks indicate statistical significance of $p < .05$ as compared to untreated samples.

Discussion

Resveratrol, a phytoestrogen found in the skins of several plants such as grapes and mulberries, could be beneficial in the treatment of multiple diseases due to its physiological effects, which include the extension of one's lifespan, cancer prevention, and, of main concern in our research, anti-inflammatory properties (Singh, Hegde et al. 2007). The damage resulting from multiple sclerosis, an autoimmune disease affecting the central nervous system, is believed to be caused by the release of certain inflammatory cytokines such as nitric oxide (NO), tumor necrosis factor-alpha (TNF- α), interleukin-12 p40 (IL-12 p40), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) which attack the myelin sheath. These cytokines have been found in the MS lesions in individuals with multiple sclerosis and are produced by activated astrocytes or microglia. These cytokines have been linked to this disease for their ability to modulate inflammation and demyelination (Benveniste 1997).

Though many treatments for multiple sclerosis are available, no drug is available that can suppress the cytokines which cause the inflammation while providing neuroprotection. Therefore, our research was centered on discovering resveratrol's effects on various cytokines linked to multiple sclerosis and its ability to provide protection to the neurons in the central nervous system attacked during an exacerbation of MS.

Our results indicate that resveratrol significantly inhibits LPS induced production of nitrite in HAPi microglial cells. To ensure that the nitrite inhibition was not caused by the compound's effect on the viability of the cell, an MTT assay was performed. The MTT assay showed that resveratrol does not affect HAPi microglial viability. Therefore,

resveratrol could suppress nitrite without killing the microglia, resulting in decreased neurodegeneration and a lightening of the symptoms associated with MS. Resveratrol was also found to suppress the pro-inflammatory cytokines IL-6, IL-12p40, TNF- α , MCP-1, and IL-1B (in primary microglia), therefore indicating resveratrol is a global suppressor of inflammatory cytokines. IL-1B is isolated from primary microglia because, after being genetically modified to divide repeatedly for its use in the lab, the HAPi cells are not able to produce this specific cytokine.

In our experiments to determine whether resveratrol provides neuroprotection, initially inducing apoptosis in the neuroblastoma cells was more arduous than had been anticipated. However, we found literature showing the ability of GeO₂ to induce apoptosis in neuro-2a neuroblastoma cells. This finding did not prove to be consistent with our research. We also felt GeO₂ was too artificial a system considering that it is a metal and not likely to be used in treatment of individuals with MS. To more closely mimic the actual environment of MS, we tried varying concentrations of IFN- γ and TNF- α . These human cytokines showed promise as well, but the results were also inconsistent. Surprisingly, in our attempts to show protection by resveratrol from apoptosis, our MTT data actually showed dose-dependent induction of apoptosis and the addition of resveratrol to GeO₂ treated cells enhanced apoptosis. Therefore, resveratrol's ability to provide neuroprotection could not be tested due to its anti-cancer ability to kill the neuroblastomas used in our experiments. Using these findings, our lab plans to test the effects of resveratrol on Ewing's sarcoma cells, another neural-derived tumor with poor prognosis, in hopes of finding a new treatment for cancer.

Bibliography

- Abemayor, E. and N. Sidell (1989). "Human neuroblastoma cell lines as models for the in vitro study of neoplastic and neuronal cell differentiation." Environ Health Perspect **80**: 3-15.
- Ascherio, A. and K. L. Munger (2007). "Environmental risk factors for multiple sclerosis. Part I: the role of infection." Ann Neurol **61**(4): 288-99.
- Barcellos, L. F., J. R. Oksenberg, et al. (2002). "Genetic basis for clinical expression in multiple sclerosis." Brain **125**(Pt 1): 150-8.
- Benveniste, E. N. (1997). "Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis." J Mol Med **75**(3): 165-73.
- Cohen, R. A. and M. Fisher (1989). "Amantadine treatment of fatigue associated with multiple sclerosis." Arch Neurol **46**(6): 676-80.
- Cresswell, P. (1998). "Proteases, processing, and thymic selection." Science **280**(5362): 394-5.
- de la Lastra, C. A. and I. Villegas (2005). "Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications." Mol Nutr Food Res **49**(5): 405-30.
- Debouverie, M., T. Moreau, et al. (2007). "A longitudinal observational study of a cohort of patients with relapsing-remitting multiple sclerosis treated with glatiramer acetate." Eur J Neurol **14**(11): 1266-74.
- Dutta, R. and B. D. Trapp (2007). "Pathogenesis of axonal and neuronal damage in multiple sclerosis." Neurology **68**(22 Suppl 3): S22-31; discussion S43-54.
- Flechter, S., J. Vardi, et al. (2002). "Comparison of glatiramer acetate (Copaxone) and interferon beta-1b (Betaferon) in multiple sclerosis patients: an open-label 2-year follow-up." J Neurol Sci **197**(1-2): 51-5.
- Freedman, D. M., M. Dosemeci, et al. (2000). "Mortality from multiple sclerosis and exposure to residential and occupational solar radiation: a case-control study based on death certificates." Occup Environ Med **57**(6): 418-21.
- Hafler, D. A., A. Compston, et al. (2007). "Risk alleles for multiple sclerosis identified by a genome-wide study." N Engl J Med **357**(9): 851-62.
- Haines, J. L., H. A. Terwedow, et al. (1998). "Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. The Multiple Sclerosis Genetics Group." Hum Mol Genet **7**(8): 1229-34.

- Holick, M. F. (2004). "Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease." Am J Clin Nutr **80**(6 Suppl): 1678S-88S.
- Imam, G. M. and H. Kuntzel (1977). "Further studies on ASN-136 and monoketo-organomycin cystaurimycin, a broad spectrum substance produced by partial enzymic digestion of monoketo-organomycin." J Antibiot (Tokyo) **30**(4): 314-20.
- Kantarci, O. and D. Wingerchuk (2006). "Epidemiology and natural history of multiple sclerosis: new insights." Curr Opin Neurol **19**(3): 248-54.
- Lu, K. T., R. Y. Chiou, et al. (2006). "Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation." J Agric Food Chem **54**(8): 3126-31.
- McQualter, J. L. and C. C. Bernard (2007). "Multiple sclerosis: a battle between destruction and repair." J Neurochem **100**(2): 295-306.
- Murray, T. J. (2006). "Diagnosis and treatment of multiple sclerosis." BMJ **332**(7540): 525-7.
- Neuhaus, O., C. Farina, et al. (2001). "Mechanisms of action of glatiramer acetate in multiple sclerosis." Neurology **56**(6): 702-8.
- Pinkston, J. B., A. Kablinger, et al. (2007). "Multiple sclerosis and behavior." Int Rev Neurobiol **79**: 323-39.
- Pollmann, W. and W. Feneberg (2008). "Current management of pain associated with multiple sclerosis." CNS Drugs **22**(4): 291-324.
- Poser, C. M. (1994). "The epidemiology of multiple sclerosis: a general overview." Ann Neurol **36 Suppl 2**: S180-93.
- Rizvi, S. A., H. Zwibel, et al. (2004). "Mitoxantrone for multiple sclerosis in clinical practice." Neurology **63**(12 Suppl 6): S25-7.
- Rudick, R. A., W. H. Stuart, et al. (2006). "Natalizumab plus interferon beta-1a for relapsing multiple sclerosis." N Engl J Med **354**(9): 911-23.
- Simioni, S., C. Ruffieux, et al. (2007). "Cognition, mood and fatigue in patients in the early stage of multiple sclerosis." Swiss Med Wkly **137**(35-36): 496-501.
- Singh, N. P., V. L. Hegde, et al. (2007). "Resveratrol (trans-3,5,4'-trihydroxystilbene) ameliorates experimental allergic encephalomyelitis, primarily via induction of apoptosis in T cells involving activation of aryl hydrocarbon receptor and estrogen receptor." Mol Pharmacol **72**(6): 1508-21.

- Sospedra, M. and R. Martin (2005). "Immunology of multiple sclerosis." Annu Rev Immunol **23**: 683-747.
- Staff, B. M. C. (2006). "Multiple sclerosis."
- Taoufik, E., V. Tseveleki, et al. (2008). "Positive and negative implications of tumor necrosis factor neutralization for the pathogenesis of multiple sclerosis." Neurodegener Dis **5**(1): 32-7.
- Whitacre, C. C. (2001). "Sex differences in autoimmune disease." Nat Immunol **2**(9): 777-80.
- Wolswijk, G. (2000). "Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis." Brain **123 (Pt 1)**: 105-15.