Soybean Supplement Therapy for Multiple Sclerosis

Proposed date of initiation of the project: July 01, 2013

Estimated Duration: one year

Summary: Multiple sclerosis (MS) is a debilitating neurological disease with an autoimmune basis. In this disease there is a loss of the insulating sheath (the myelin) of the impulse conducting part of the nerve cell (the axon) as well as loss of the axons themselves. Largely ignored and discounted by mainstream medicine, nutrients offer immune-modifying benefits that can complement pharmacological and clinical interventions and improve quality of life for MS patients. Whole soybeans contain substances which have been shown to provide protection and improve the clinical outcome in animal models of MS. The objective of this study is to determine the potential of soybean therapy in a mouse model for MS called experimental autoimmune encephalomyelitis (EAE). This dietary intervention could be shown to be as efficacious as some of the currently available drugs and it would be a major advance for the MS patients and it will also lead to increased sales of soybeans. We will investigate if dietary supplementation with dehulled soybean meal in a chronic EAE model will significantly reduce the incidence, delay the onset and reduce the severity of disease. To achieve this goal, C57BL/6 female mice will be placed on either a control diet or soy-supplemented diets. The diets will be administered at three different time points: prior to onset of disease to test if the diets will significantly delay the onset and lessen the severity of the disease, early in the course of disease and at the peak of disease to assess for the ability of these dietary supplements to reverse or stabilize established disease. All cohorts of mice will then be compared with respect to EAE scores. In addition, morphological studies will be carried out on the diseased and soybean-treated animals to determine how much myelin is lost and the degrees to which the loss of nerve processes (axons) is prevented by soybean treatment. If our hypothesis is correct, the soybean-fed mice should be less affected by EAE when compared to the control-fed mice, have significantly lower clinical scores as well as significantly greater myelin and axonal preservation in the lumbar spinal cord and less infiltration of immune cells into the nervous tissue.
Objectives -- Multiple sclerosis (MS) is a debilitating neurological disease with a strong autoimmune and inflammatory component. Although a number of drugs are now available which slow the progression of disease, the drugs are expensive and not readily available to the majority of MS patients. The animal model of MS is experimental autoimmune encephalomyelitis (EAE), a model which mimics pathological and clinical features of MS, making it amenable for mechanistic and intervention studies, (1-4). Whole soybeans contain substances which have been shown to have immunomodulatory, anti-inflammatory, and neuroprotective properties. The objective of this study is to determine the degree to which soybeans will provide protection in EAE. This dietary intervention could be then tested as a readily available therapy in MS.

Justification/Practical Importance -- Currently, MS affects over 400,000 individuals in the United States and about 2.1 million individuals worldwide. If a soybean dietary intervention could be shown to be efficacious, it would provide a cost effective therapy accessible to the majority of the MS patients. If the majority of the affected MS patients were to use the soybean therapy it would generate an estimated increased demand of 1600 tons of soybeans per year in the USA. In turn, if this therapy is extended to the worldwide population of MS patients it would generate additional sales of about 8,000 tons of soybeans per year. More importantly, since there have been virtually no studies of the effect of soy on neurological status, the proposed study would provide a sound basis to study the role of soy in other neurological diseases such as Alzheimer’s and Parkinson’s disease.

Background -- Largely ignored and discounted by mainstream medicine, nutrients offer immune-modifying benefits that can complement pharmacological and clinical interventions and improve quality of life for MS patients. For example, in a rat model of Parkinson’s disease, supplementation with dietary blueberry extract increased the survival of implanted dopaminergic neurons, consequently diminishing the neuronal damage cause by the disease, (5). Also, dietary blueberry supplementation improved cumulative and final motor scores in a mouse model of EAE (6) and more recently, it has been shown to improve the outcome of traumatic brain injury as well (McGuire - personal communication).
Soybeans are an abundant source of a class of compounds known as isoflavones. The major soybean isoflavone is genistein which constitutes 50% of the total isoflavone content, (7-10). Isoflavones are a class of molecules called flavonoids that belong to a large family of polyphenols. Our recent studies of a polyphenol (Didox) have shown that it can delay the onset and severity of EAE and reverse the course of established disease. Polyphenols may reduce inflammation and neuronal damage by modulating the immune response, (11) and decreasing the ability of immune cells to infiltrate into the central nervous system (CNS), (12). As shown by De Paula et. al., sub-cutaneous injection of one of the active components of soybeans, genistein, ameliorated the course of EAE by inhibiting the clinical symptoms of disease as well as the pro-inflammatory cytokine secretion, (13). Even though the individual components of soybeans have been shown to be effective, no one has yet evaluated the effects of whole soybean meal in any animal model of degenerative disease. This study will show for the first time the degree to which whole soybean in the diet can modulate the course of this MS mimicking disease.

**Procedure** -- We will investigate if dietary supplementation with dehulled soybean meal in a chronic EAE model will significantly reduce the incidence, delay the onset and reduce the severity of disease. The ability of the soybean supplementation to reverse established disease in EAE will also be evaluated. The regular rodent chow has a minimum 5% inclusion of soybeans, so we designed the experiments in a soybean dose-dependent manner, including a soybean percentage lower than the standard diet. The diets will be administered at three different time points: prior to onset of disease to test if the diets will significantly delay the onset and lessen the severity of the disease, early in the course of disease and at the peak of disease to assess for the ability of these dietary supplements to reverse established disease. The chow will be provided ad libitum and replaced every day.

**Diet Preparation:** Diets will be prepared by Harlan Teklad as follows: AIN-93M will be used as a fixed-formula purified control diet containing 12.4% protein, 4.1% fat and 68.4% available carbohydrate and supplemented with standard mineral and vitamin mixes. The soy-supplemented diet will be made by replacing 3%, 6%, 9% and 12% of the ground corn (w/w) in the control diet with 3%, 6%, 9% and 12% dehulled soybean meal, (Table 1). All diets will be calculated to be isocaloric and made from a common basal mix at the same time, thus
differing only in soybean meal supplementation. C57BL/6 female mice will be placed on AIN-93M chow until 3 days before the beginning of the immunization protocol. At that point, mice will be randomly assigned to one of the five dietary treatment groups, the fixed-formula control AIN-93M chow (non-supplemented diet, cohort 1) or the soy-supplemented chows (cohorts 2-5). These experimental diets will continue throughout the study and all cohorts of mice will be compared with respect to EAE scores. Moreover, immunohistochemistry and morphological studies will be performed, so animals will be sacrificed at various disease stages and perfused to assess for the degree of demyelination, axonal preservation and immune infiltration.

**Animals and Induction of EAE:** To induce chronic EAE, all mice groups will be injected with a myelin peptide (myelin-oligodendrocyte glycoprotein or MOG35-55) followed by a pertussis toxin intraperitoneal injection to mimic demyelinating disease. This method has been successfully used in our lab for several years. We will be using C57 BL/6 female, 11 weeks old mice for this model. Mice will be monitored daily and clinical signs of disease will be scored from day seven after immunization. The severity of impairment due to EAE will be measured in all experiments by the following a scoring system: 1, limp tail; 2, impaired righting reflex; 3, one hind limb paralysis; 4, both hind limbs paralysis; 5, quadriplegia or pre-moribund state. The mice typically begin to show signs of EAE a week after the MOG injection, reaching an average clinical score of about 3 in 14 days. The sample size required to observe a statistical difference between different dietary treatment groups undergoing EAE is 12 animals. EAE group size is based on data from previous experience of our lab with the EAE mouse model. The average standard errors are expected to be 30%-40% of the average maximal clinical score (n=40). In order to detect a 15% effect at significance level of 0.95 and a power of 0.80, a group size of minimum 12 is required. Cohorts will be compared on the parameters of incidence, severity and onset of disease and related to the extent of demyelination. At the conclusion of the experiment 3 animals from each group will be perfused, spinal cords and brains will be removed and examined for extent of immune infiltration, myelin integrity and extent of axonal loss.

If our hypothesis is correct, the soybean-fed mice should be less affected by EAE when compared to the control-fed mice, have significantly lower clinical scores as well as significantly greater myelin and axonal preservation in the lumbar spinal cord and less immune infiltration.
Table 1. Disposition of Cohorts of Mice

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Strain/Age</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control non-supplemented AIN-93M diet</td>
<td>C57BL/6, 11 weeks</td>
<td>12</td>
</tr>
<tr>
<td>2. Exp. 3% soybean-supplemented diet</td>
<td>C57BL/6, 11 weeks</td>
<td>12</td>
</tr>
<tr>
<td>3. Exp. 6% soybean-supplemented diet</td>
<td>C57BL/6, 11 weeks</td>
<td>12</td>
</tr>
<tr>
<td>4. Exp. 9% soybean-supplemented diet</td>
<td>C57BL/6, 11 weeks</td>
<td>12</td>
</tr>
<tr>
<td>5. Exp. 12% soybean-supplemented diet</td>
<td>C57BL/6, 11 weeks</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total Number of Animals</strong></td>
<td></td>
<td><strong>60</strong></td>
</tr>
</tbody>
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* Experiments will be repeated 3 times, at three time points so the total number of mice is 540.

References –
Personnel/Facilities -- Andreea Marcu, M.D. will perform all experiments. She has worked with rodent models for the last nine years and is well trained in the EAE model. All experiments will be take place at the McGuire Research Institute (MRI) facility in Richmond, VA.

Other Entities -- McGuire Research Institute will solely administer the grant.

Source of Other Funds -- No other funding source will be used to complete this project. This is a pilot grant that could lead to significant support for future research.

Percentage of Funding -- 100% of the animal and lab reagents costs would be funded by the soybean checkoff and 50% of salary and fringe for Andreea Marcu.

Budget --

MOG peptide (250 mg x $20.24) $ 5,060
Pertussis Toxin (4 vials of 50 ug x $250) $ 1,000
Friends Adjuvant (40 ml) $ 130
C57/Bl6 mice (540 mice x $23.85) $12,879
Housing the mice (540 mice x $ 0.4 x 30 days) $ 6,480
Soybean diets and packaging (5 diets of 3kg each x $183) $ 915
Immunohistochemistry Antibodies and Reagents $ 1,636
Immunohistochemistry Scope Hours (20 hours x $5) $ 100
Confocal Microscopy (20 hours x $25) $ 1,000
Electron Microscopy (20 hours x $40) $ 800
Electron Microscopy Processing (30 mice x $250) $ 7,500
Salary and fringe for Andreea Marcu (50%) $27,500
Indirect costs for MRI (20%) $13,000

Total project cost $78,000

Equipment -- No equipment will be purchased from this grant.

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