Treatment of Schizophrenia

The present application is based on, and claims priority from United States Application No. 60/270,087 filed February 20, 2001.

Background of the Invention

Schizophrenia is a common, complex, heterogeneous, adult onset, polygenic neuropsychiatric disease. Schizophrenia affects 1-1.5% of the population and is a major cause of mortality and morbidity. Although clearly heritable, the disease follows a non-Mendelian pattern of inheritance. One theory posits that schizophrenia is a caused by neuro-degenerative CAG trinucleotide repeats encoding polyglutamine arrays typically longer than 35 repeats in the coding regions, or the untranslated sequences (introns) of genes. These polyglutamine repeats modulate gene or protein structure and function.

Pathophysiological models of the disease have localized its expression to the dopaminergic neurons of the mid-brain—the substantia nigra and ventral tegmental area—from these dopaminergic neurons into the nigrostriatal and mesolimbic pathways. These two pathways encompass the very limited region of the central nervous system (CNS) that expresses Dopamine D2 receptors. Imaging and functional studies have previously implicated these two pathways in the pathogenesis of schizophrenia. The human hippocampus and amygdala are two regions also implicated in effect and psychosis. All of these regions show the presence of a novel human cDNA sequence that encodes a neuronal small conductance calcium-activated potassium channel (hSKCa3). This nucleotide has been isolated

and sequenced. Small conductance calcium activated potassium (K^+) channels play a critical role in determining the firing pattern of neurons.

The mis-match expansion of genes by DNA repair proteins during meiosis leads to a misconstruction of 144 glutamine residue arrays (polyglutamines) in these ion channel proteins. The polyglutamine repeats alter the hSKCa3 channel function and neuronal excitability and thereby increase disease risk when combined with other genetic and environmental factors. Northern-blot analysis has revealed hSKCa3 specific expression to be largely limited to brain, striated muscle, and lymphoid tissues. Small conductance channels (SKCa) are known to play a vital role in determining the firing pattern of neurons via the generation of slow after hyperpolarization and in regulating of cellular calcium signals. Alteration in hSKCa3 channel function might be expected to affect neural excitability. SKCa channels hyperpolarize the membrane potential of neurons and thereby inactivate NMDA-R receptors (N-methyl-o-aspartate type glutamate receptor channels). The NMDA-R receptors are the major excitatory receptors in the central nervous system. Hyperactive hSKCa3 channels would therefore be expected to induce NMDA-R receptor hypofunction and might thereby enhance susceptibility to disease.

When bound by an agonist, the NMDA-R receptor channels allow calcium and sodium to be transduced by potassium channels in a inward current. There are interactions between the activity of voltage-gated calcium channels (products of the alpha-1 calcium channel gene), calcium activated potassium channels (hSKCa3) and NMDA-R receptors. Each channel has the potential to control the activity of the other. Alteration of function in any one of these important proteins might perturb a common physiological process. The hippocampus and the amygdala have demonstrated an abundant expression of hSKCa3. This hSKCa3 is expressed in dopaminergic

neurons of the dopaminergic pathways of the nigrostriatal and mesolimbic pathways. Fluorescence *in-situ* hybridization (FISH) analysis shows that hSKCa3/KCNN3 is located on chromosome 1q21. Longer alleles are associated with more negative symptom dimension scores of the schizophrenic phenotype (P= .01). CAG repeats modify the function of the hSKCa3 channel.

The biophysics of the channels has become better characterized. The novel, high affinity calcium site involved in activation has been shown to be calmodulin. In a comprehensive analysis of all patients in a very homogeneous Finnish population, which can be traced back over 300 years, a genome-wide scan identified chromosome 1q32 as a contributing region (marker spacing of 11cM). Familial hemiplegic migraine locus was mapped to 1q21, a CAG - containing calcium gene involved in SCA6 and familial migraine.

Based upon studies in more homogeneous Israeli-Ashkenazi Jews, a highly significant p-value (P= 0.00017, Wilcox Rank Sum test) for the disease association were multi CAG repeat alleles. In all, fifty-nine parent/offspring trios were used. In these groups hSKCa3 contained two arrays of CAG trinucleotide repeats. It was found in the group that the second CAG repeat was highly polymorphic in control individuals, with alleles ranging in size from 12 to 28 repeats. The overall allele frequency distribution is significantly different in patients with schizophrenia compared to ethnically matched controls (Wilcox Rank Sum test, P= 0.024) with CAG repeats longer than the model value being over-represented in patients (Fisher Exact test, P= 0.0035). Modal value was 19.

The hSKCa3 gene encodes a protein of 731 amino acids and is exquisitely sensitive to cytosolic free calcium. The rise in calcium via

NMDA-R and ligand binding of glutamate, causes the hSKCa3 gene to send a small conductance of 4-14 pS (pico Siemens) to after-hyperpolarize the membrane and set up Kv (voltage gated potassium channel) to further polarize the membrane with 12-40pS. This also sets the tonic magnesium (Mg^{2+}) block into the NMDA-R channel stopping the Ca^{2+} pumping. Depolarization starts this process all over again in sub-milliseconds. Channel blockers, such as ketamine, phencyclidine (PCP) and MK 801 block NMDA-R channels and induce schizophrenia-like syndromes. Conversely, many antipsychotic drugs exert an activating agonist-like effect on NMDA-Rs. Based on these finding it has been suggested that a reduction in the activity of NMDA-R signaling pathway would contribute to the pathophysiology of schizophrenia. Greater potassium channel activity results in a decreased discharge frequency. Drugs designed to block this channel should treat neuropsychiatric disease, since this gene has a highly restricted pattern of expression in the human brain and is capable of modulating neuronal excitability through the generation of slow after-hyperpolarizations. Increased potassium channel activity would indirectly cause the reduction in activity of the NMDA-R via an extracellular Mg^{2+} block. Thereby the pathophysiology of schizophrenia would be exacerbated. Drugs like valproate and lithium stimulate glutamate release and activate NMDA-Rs.

A novel therapeutic would be a small regulatory molecule whose function would be to potentiate the ion channel by way of sequestering calcium. This would allow the ion channel to have greater load electrochemically to hyperpolarize and thereby maintain the NMDA-Rs open by delaying onset of the tonic magnesium block. This would maintain the cytosolic free calcium potentiating the hSKCa3 channel itself to fire less often. Keeping the NMDA-R open would allow neural excitability and relief of psychosis. Conversely, hyperactive hSKCa3 channels would be expected

20 JUN 2005

to induce NMDA-R receptor hypofunction and thereby enhance disease susceptibility.

Enzyme active site modeling and X-ray crystallography have shown that flavones with specific hydroxylation patterns can cause calcium sequestration in signal transduction pathways. Receptors r3 and r4 of the thyroid hormone family are inhibited by ligand binding of hydroxlylated flavones, thereby deactivating the oxygen transport hormone and therefore oxygen transport. The polyglutamine arrays (CAG repeats) can produce a increased affinity in the hSKCa3 gene channel due to the increased accumulated potential. These additional arrays can be combated by increased Ca^{2+} loading in the signal transduction pathways. Application of ligand specific flavonoids would draw calcium to the S5 and S6 pore or p region to keep the NMDA-R channel open. Furthermore, the hSKCa3 gene product would need to modulate less frequently due to calcium cytosolic loading. This potentiation would cause less frequent after-hyperpolarization leading to less tonic blocking of the NMDA-R by Mg^{2+}, thereby increasing calcium flow for further hypofuction.

Summary of the Invention

The present invention involves the use of flavonoids for the production of a pharmaceutical composition for the treatment of schizophrenia. I have discovered that flavonoids act as the ideal modulators of hSKCa3 ion channels. When schizophrenia patients are treated with oral doses of common flavonoids such as quercetin, luteolin and myricetin, the symptoms of the disease are dramatically ameliorated. Many of the effective flavonoids are common in a number of fruits and vegetable. However, normal dietary intake does not appear to provide sufficient flavonoids for therapeutic action.

Detailed Description of the Invention

Flavonoids are ubiquitous chemicals found in many vascular plants. Flavonoids are a well-studied group of chemicals and the standard system of numbering and nomenclature are used in the following discussion. A variety of different structural groups are found in differing configurations on the three rings of a flavonoid. Additionally, organic chemists can add many new and non-naturally occurring structural groups to create novel flavonoids. Key structural components in the flavonoid molecule which impart functionality to effectively treat schizophrenia are sites for the complexing of Ca^{2+} created by hydroxyl groups and other electron donating atoms or groups including but not limited to methoxy, ethoxy, amino, amido, hydroxyethyl, halogens, sulfuryl and sulfhydryl, and phosphoryl groups.

Of specific interest in the present invention are the flavones quercetin, luteolin, myricetin, hesperetin, apigenin, baicalein, naringenin, and the many glycosides and methyl ethers thereof including but not limited to rutin, diosmin, hesperidin, myricitrin, etc. These naturally occurring flavones and their glycosides, by complexing with and sequestering Ca^{2+}, help stabilize the charge of the over built and misconstructed CAG repeats. Too many CAG (over 19) alleles (Fisher Exact test, $P = 0.0035$) cause the NMDA-R to operate improperly, resulting in psychosis. It is therefore thought that by stabilizing the charge density of the overbuilt and misconstructed CAG repeats the flavonoid molecule restores function to the NMDA-R and therefore reduces the symptoms associated with schizophrenia.

Effective treatment has been achieved with quercetin as well as with mixtures of flavonoids—primarily luteolin, quercetin, and myricetin, but other flavonoids show activity. It has not yet been determined which

flavonoids or mixtures of flavonoids are the most effective. The therapeutic ability of a flavonoid or a flavonoid mixture can be estimated by measuring the ability of a test material to alter the *in vitro* functioning of the hSKCa3 ion channels. Although flavonoids are common components in human diets, it does not appear that normal diets contain adequate concentrations of flavonoids to act therapeutically. It remains to be determined, but it seems reasonable to suppose that in some cases dietary flavonoids are adequate to suppress the onset of schizophrenia and related psychoses that are mediated through the hSKCa3 ion channels. Dietary variations may help explain the variability seen in schizophrenia onset in identical twin experiments. Perhaps the old adage should be reformed to read: "An apple a day keeps the psychiatrist away."

Examples of the Invention

Subject number one, M.R, was 21-22-year-old paranoid schizophrenic male. He exerted classic symptoms of schizophrenia: he would not come out of his room; he would not converse with his family or parents; and he reported hearing voices coming out of the pipes in his bedroom. He had gained approximately 35+ pounds while receiving classic neuroleptics, and anti-psychotic drugs to treat his symptoms. His speech was slow, and he would not look directly at the interviewer. His thought process seemed unfocused and scattered. His gait was "stuttered" showing small baby steps. His weight was approximately 230 pounds, and he had dark circles under his eyes.

M.R.'s parents were unable to care for him and sought to permanently institutionalize him. I started M.R. on a 500mg 3x/day oral dose regime of quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone). After 30 days of treatment with quercetin, the subject no longer heard voices, began

communicating with his family, left his room, lost weight, lost the dark circles under his eyes, and became rational in his thought processes. The administration of quercetin had a marked, demonstrable effect on reducing the symptoms of paranoid schizophrenia.

Subject number two, M.F., was a 44-year-old female and had been diagnosed with Lupus about five years before the time of treatment. Lupus is often associated with dementia. Probably in relation to that disease, M.F.'s emotional status began to decline severely. Her husband took her to psychiatric and marriage counseling and became discouraged to the point of contemplating divorce. He felt that his wife was lost to him and "gone" emotionally. After thirty days of treatment with the mixed quercetin and myricetin (1500 mg/day), she regained composure, and her husband stated that she had returned to him. The irrational behavior and psychosis's abated and counseling was terminated.

Subject number three, D.S., was a 42-year-old Caucasian female. D.S. was arrested for shop lifting from model homes. She did this in front of the managers of the site and asked employees of the construction company to come and assist her in loading her car with paintings and typewriters so she could take them home. Needless to say, this bizarre behavior surprised the personnel guarding the model homes who promptly called the police. Meanwhile, D.S. strolled from model home to model home looking for merchandise to take. She was quite shocked and disoriented when the police arrested her on the premises. This behavior occurred several times.

After taking oral flavonoids (primarily quercetin) for two months, the probation attorneys reported that her bizarre behavior had ceased. D.S.'s husband reported that prior to flavonoid treatment she would speak out loud to unseen persons and converse with them during dinner. This so distressed

D.S.'s children that they had to leave the table. Since taking the flavonoid, this strange behavior completely disappeared.

The following claims are thus to be understood to include what is specifically illustrated and described above, what is conceptually equivalent, what can be obviously substituted and also what essentially incorporates the essential idea of the invention. Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiments can be configured without departing from the scope of the invention which is the treatment of schizophrenia and related psychoses by the administration of flavonoids. The illustrated embodiment has been set forth only for the purposes of example and that should not be taken as limiting the invention. Therefore, it is to be understood that, within the scope of the appended claims, the invention may be practiced other than as specifically described herein.

Claims

What is claimed is :

1. The use of a flavonoid for the production of a pharmaceutical composition for the treatment of schizophrenia.

2. The use according to Claim 1, wherein the pharmaceutical composition is orally administered.

3. The use according to Claim 1, wherein the flavonoid is a naturally occurring flavonoid selected from the group consisting of quercetin, hesperetin, luteolin, myricetin, apigenin, baicalein, and naringenin.

4. The use according to Claim 1, wherein the flavonoid is a glycoside, ester or conjugate of a flavonoid.

5. The use according to Claim 5, wherein the glycoside is selected from the group consisting of rutin, diosmin, hesperidin, and myricitrin.

6. The use of a quercetin for the production of a pharmaceutical composition for the treatment of schizophrenia.

7. The use according to Claim 6, wherein the pharmaceutical composition is orally administered.